Twitter Thread by Very 0wn

Very 0wn
@Very_0wn

“John D. Rockefeller is credited as a founder of Pharmaceutical Industry.”

|

“Beginning in 1930, the Rockefeller Foundation provided financial support to the Kaisier Wilhelm Institute, Human Heredity, and Eugenics, which later conducted eugenics experiments in the Third Reich.”

> Rockefeller and Big Pharma...https://t.co/E5ZlrYYQXm pic.twitter.com/z5wKflhimT
>
> — Shorty (@Shorty56167141) August 21, 2021

IRS Copy of 990-PF form of The Rockefellers Brothers Fund (2001):
https://t.co/ZH0zo7Lc6b
Grant to "Eleanor Roosevelt Institute Tor Cancer Research - To the research of Kathleen Gardiner, PhD, entitled "A-to-l mRNA Editing of Mammalian Genes Relevance to Learning and Behavior".

IRS COPY

Form **990-PF**

Department of the Treasury
Internal Revenue Service

Return of Private Foundation
or Section 4947(a)(1) Nonexempt Charitable Trust Treated as a Private Foundation

Note: *The organization may be able to use a copy of this return to satisfy state reporting requirements*

OMB No 1545-0052

2001

For calendar year 2001, or tax year beginning , 2001, and ending

G Check all that apply: Initial return | Final return | Amended return | Address change | Name change

Use the IRS label. Otherwise, print or type. See Specific Instructions.

Name of organization: THE ROCKEFELLER BROTHERS FUND, INC

A Employer identification number: 13-1760106

Number and street (or P O box number if mail is not delivered to street address): 437 MADISON AVENUE 37TH FLOOR

Room/suite:

B Telephone number (see page 10 of the instructions): (212) 812-4200

City or town, state and ZIP code: NEW YORK, NY 10022-7001

C If exemption application is pending, check here ▶

D 1 Foreign organizations, check here ▶

2 Foreign organizations meeting the 85% test, check here and attach computation ▶

E If private foundation status was terminated under section 507(b)(1)(A), check here ▶

F If the foundation is in a 60 month termination under section 507(b)(1)(B), check here ▶

H Check type of organization: [X] Section 501(c)(3) exempt private foundation
[ ] Section 4947(a)(1) nonexempt charitable trust [ ] Other taxable private foundation

I Fair market value of all assets at end of year (from Part II, col (c), line 16) ▶ 684,464,383

J Accounting method: [ ] Cash [X] Accrual [ ] Other (specify)
(Part I, column (d) must be on cash basis)

Part I Analysis of Revenue and Expenses (The total of amounts in columns (b), (c), and (d) may not necessarily equal the amounts in column (a) (see page 10 of the instructions))

		(a) Revenue and expenses per books	(b) Net investment income	(c) Adjusted net income	(d) Disbursements for charitable purposes (cash basis only)
Revenue	1 Contributions, gifts, grants, etc. received (attach schedule) . Check ▶ [ ] if the foundation is not required to attach Sch B	282,834	STMT 1A		
	2 Distributions from split-interest trusts				
	3 Interest on savings and temporary cash investments	1,357,768	1,357,768		
	4 Dividends and interest from securities	13,181,361	17,256,969		
	5a Gross rents				
	b (Net rental income or (loss) _____)				
	6a Net gain or (loss) from sale of assets not on line 10	-24,502,865			
	b Gross sales price for all assets on line 6a _____				
	7 Capital gain net income (from Part IV, line 2)		NONE		
	8 Net short-term capital gain				
	9 Income modifications				
	10a Gross sales less returns and allowances _____				
	b Less Cost of goods sold _____				
	c Gross profit or (loss) (attach schedule)				
	11 Other income (attach schedule) STMT 1	140,373	-2,181,136		
	12 Total Add lines 1 through 11	-9,540,529	16,433,601		
Expenses	13 Compensation of officers, directors, trustees, etc	1,071,959	157,478		914,481
	14 Other employee salaries and wages	2,396,742	39,347		2,357,395
	15 Pension plans, employee benefits	1,412,074	79,958		989,136
	16a Legal fees (attach schedule) STMT 2	174,096	8,096		119,581

RECEIVED
NOV 10 2002
OGDEN, UT

Gene regulation by mRNA editing (1995):
https://t.co/K2H1rc2Uvx

"Despite the potential power of mRNA editing to generate new and useful gene products, it is clear that it must be tightly regulated. Unrestricted mRNA processing could lead the cell to synthesize toxic proteins..."

Am. J. Hum. Genet. 60:278–283, 1997

INVITED EDITORIAL
Gene Regulation by mRNA Editing

John Ashkenas

The American Journal of Human Genetics

The commonly cited figure of 10^5 genes in the human genome represents a tremendous underestimate of our capacity to generate distinct gene products with unique functions. Our cells possess an impressive collection of tools for altering the products of a single gene to create a variety of proteins. The different gene products may have related but distinct functions, allowing cells of different types or at different developmental stages to fine-tune their patterns of gene expression. These tools may act in the cytoplasm, as when proteins undergo post-translational modifications, or in the nucleus, in the processing of pre-mRNA.

Two forms of intranuclear fine-tuning are well established and widely studied: alternative splicing of pre-mRNAs and alternative polyadenylation site selection. In recent years it has become clear that cells possess yet another tool to create RNA sequence diversity, mRNA editing. The term "editing" is applied to posttranscriptional modifications of a purine or pyrimidine, which alter an mRNA sequence as it is read, for example, by ribosomes. Covalent changes to the structure of nucleotide bases are well known to occur on tRNA and rRNA molecules, but such changes in mRNA sequence are novel in that they have the capacity to change specific protein sequences.

Despite the potential power of mRNA editing to generate new and useful gene products, it is clear that, like splicing and polyadenylation, it must be tightly regulated. Unrestricted mRNA processing could lead the cell to synthesize toxic proteins or to make an otherwise useful protein at the wrong time. The control of these functions is only beginning to be understood, but there are fascinating hints that the different classes of intranuclear pre-mRNA processing are coordinated. As our understanding of these events matures, we may come to see them each as different aspects of a single RNA-processing mechanism.

Received December 4, 1996; accepted for publication December 6, 1996.

Address for correspondence and reprints: Dr. John Ashkenas, *The American Journal of Human Genetics*, Department of Pathology, University of Washington, Seattle, WA 98195-7470. E-mail: jayemay@u.washington.edu

This article represents the opinion of the author and has not been peer reviewed.

0002-9297/97/6002-0006$02.00

Forms of mRNA Editing

To date, four classes of mRNA editing have been observed in mammalian cells (fig. 1); it seems unlikely that this list is complete. Because it has not been possible to search systematically for discrepancies between mRNA and genomic sequences, the list of mRNAs known to be edited is short, but it will almost certainly expand as this novel aspect of gene regulation is explored.

C-to-U Editing and the ApoB mRNAs

The first recognized and best-understood example of editing in mammalian cells occurs in epithelial cells of the small intestine, enterocytes. These cells synthesize a form of apolipoprotein B, ApoB-48, and insert it into chylomicrons, the carrier particles that allow dietary lipid to circulate in the bloodstream. Human liver cells express the identical gene, *ApoB*, but they synthesize the larger protein, ApoB-100, found in another class of lipid carrier, the LDL particle. ApoB-48 and ApoB-100 peptide sequences are identical at their N-termini, but ApoB-48 terminates at 48% of the length of the longer protein. A single base difference accounts for a break in the open reading frame in the ApoB-48 mRNA. Nucleotide 6666 in ApoB-100 is the C in the glutamine codon CAA; the corresponding nucleotide in ApoB-48 is a U, forming the stop codon UAA. This UAA is not encoded in any genomic copy of *ApoB*. As first suggested nearly a decade ago, U6666 is the product of a covalent change in ApoB mRNA sequence (Chen et al. 1987; Powell et al. 1987; Higuchi et al. 1988).

Boström et al. (1990) developed an in vitro mRNA-editing assay and demonstrated that the C-to-U modification involves the direct deamination of cytosine to form uracil (fig. 1), and they found this enzymatic activity in lysates from a variety of cells, including several that do not express the *ApoB* gene. The sequence specificity of editing is directed by an 11-nucleotide sequence (described as the mooring sequence) located 5 nucleotides 3′ of a cytosine; this sequence is sufficient to allow some heterologous mRNA species to be edited. The efficiency of editing also depends on other *cis*-regulatory

mRNA mediates passive vaccination against infectious agents, toxins, and tumors (2017):
https://t.co/Fj6ZNU7DjA

Enlisting the mRNA Vaccine Platform (2019):
https://t.co/XqjKygr2yR

The paper explained

Problem

While antibodies offer a potent and widely used means in cancer treatment, there are only three approved monoclonal antibodies against infectious diseases today, although protective antibodies have been described for a variety of pathogens. Thus far, antibodies are at a disadvantage compared to antibiotics and vaccines, predominantly for economic reasons. Hence, there is a strong demand for cost-effective alternatives to the administration of recombinant proteins. DNA-based antibody expression proved to reach protective serum titers in various preclinical models. However, permanent or at least long-lasting expression is often undesired for passive immunization, thus requiring sophisticated approaches for the clinic. By contrast, transience is inherent to another nucleic acid, mRNA. Moreover, a number of recent intriguing studies principally suggested mRNA as a means for molecular therapies requiring the delivery of functional proteins.

Results

In vitro and *in vivo* experiments revealed efficient expression of functional antibodies from exogenous mRNA. Flexibility of the technology was demonstrated by the expression of different antibodies and antibody formats such as classical IgG-based monoclonal antibodies (mAbs) or heavy-chain-only neutralizing agents (VNAs). *In vivo*, LNP–mRNA-mediated antibody expression protected mice against diverse biological threats such as virus, toxin, and neoplastic cell growth. Protection was obtained in pre- and post-exposure treatment scenarios. mRNA revealed a favorable expression kinetics giving rise to immediately elevated antibody serum titers. As a consequence, the efficacy of mRNA was comparable to recombinant antibody therapy in a particularly challenging post-exposure scenario for intoxication.

Impact

Today, passive immunization only fills a niche in preventing or fighting infectious diseases, mainly due to non-competitive costs compared to antibiotics and vaccines. However, there is renewed interest in passive immunization, for instance since the emergence of microbial resistance to antibiotics increased the demand for alternative therapies. The present study reveals that mRNA-mediated antibody expression can confer protection against diverse biological threats. We suggest formulated mRNA as a novel armamentarium for the development of competitive passive immunization therapies.

Reverse-transcribed SARS-CoV-2 RNA can integrate into the genome of cultured human cells and can be expressed in patient-derived tissues (December 2020):

https://t.co/aY00nqMv5b

'Protecting' from endothelial bio-weapon by leaky temporary mRNA gene-therapy...

Strongest Evidence Yet Shows SARS-CoV-2 May Insert Itself Into The Human Genome

MIKE MCRAE 12 MAY 2021

Our genome is a graveyard littered with genetic fragments of viruses that once plagued our ancestors. If a controversial claim by MIT researchers withstands the criticisms being leveled at it, the virus behind the current pandemic has a fair chance of joining them.

Having a few chunks of virus code scattered among our genes doesn't necessarily mean the pandemic is here to stay. It could even go some way towards explaining why a handful of patients continue to test positive for COVID-19 long after recovery.

But SARS-CoV-2 simply isn't equipped with the tools to bury itself in our genetic library, meaning it would need a way to convince our own bodies to manage the job on its behalf.

"SARS-CoV-2 is not a retrovirus, which means it doesn't need reverse transcription for its replication," says biomedical researcher Liguo Zhang from MIT's Whitehead Institute.

"However, non-retroviral RNA virus sequences have been detected in the genomes of many vertebrate species, including humans."

Last year, Zhang and his team shared the initial results of an investigation suggesting SARS-CoV-2 might have a means of accomplishing such a task after all.

Using published data sets of infected cell cultures and patient samples, the team identified part-human, part-virus transcripts among sequences produced by the cells.

This was followed by experiments that assessed whether the presence of SARS-CoV-2 particles was enough to stimulate cells into producing certain enzymes that specialize in reverse transcribing RNA into DNA.

Their findings supported the rather concerning possibility that sequences of the coronavirus could be copied and pasted into our genome; importantly, not everybody in the scientific community was convinced by the evidence.

The Rockefeller University and Broad Institute of MIT and Harvard announce update to CRISPR-Cas9 portfolio filed by Broad (January 2018):
https://t.co/rn96X5IOlk
"CRISPR-Cas9 system - Drs. Marraffini and Feng Zhang are co-inventors and Rockefeller and Broad are joint owners."

PRESS RELEASES / 01.15.18

EMAIL

The Rockefeller University and Broad Institute of MIT and Harvard announce update to CRISPR-Cas9 portfolio filed by Broad

An update regarding inventorship and ownership of certain Broad filings relating to the use of the CRISPR-Cas9 system in eukaryotic cells

The Rockefeller University - Researchers use new CRISPR-based strategy to replicate disease in cells (May 2016):
https://t.co/usr9s8ECWX
- Making gene editing more reliable

"mRNA is a way to make CRISPR gene editing come alive. CRISPR is the workhorse; mRNA encodes it."

THE ROCKEFELLER UNIVERSITY
Science for the benefit of humanity

Researchers use new CRISPR-based strategy to replicate disease in cells

May 4, 2016

To explore in detail how specific genetic errors can lead to disease, scientists need to perform experiments in cells that carry these exact mutations. Now, the ability to create these cellular replicas using new genome editing technology has been facilitated thanks to work by Rockefeller University researchers.

Out of body: Researchers in the Tessier-Lavigne lab use human brain neurons (above) derived from stem cells to study dementia. A new approach developed in the lab allows them to more efficiently introduce mutations associated with diseases, such as Alzheimer's.

Luciano Marrafini, the Rockefeller University, - Harnessing CRISPR-Cas9 immunity for genetic engineering (2014)
https://t.co/uwmVabxbke

CRISPR's many pioneers (2020):
https://t.co/NXcBrh6wbb

Jennifer Doudna, WEF Agenda Contributor, - CRISPR in 4IR:
https://t.co/XuByFKbzJg

Harnessing CRISPR-Cas9 immunity for genetic engineering

Emmanuelle Charpentier [1, 2, 3] ✉, Luciano A Marraffini [4] ✉

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https://doi.org/10.1016/j.mib.2014.07.001

Highlights

- CRISPR-Cas is an RNA-mediated adaptive immune system against mobile genomes.
- RNA-programmable CRISPR-Cas9 enables targeted genome editing.
- Catalytically inactive CRISPR-Cas9 allows targeted modulation of transcription.
- Cas9 can be fused to diverse functional domains to manipulate and modify DNA.

CRISPR-Cas encodes an adaptive immune system that defends prokaryotes against infectious viruses and plasmids. Immunity is mediated by Cas nucleases, which use small RNA guides (the crRNAs) to specify a cleavage site within the genome of invading nucleic acids. In type II CRISPR-Cas systems, the DNA-cleaving activity is performed by a single enzyme Cas9 guided by an RNA duplex. Using synthetic single RNA guides, Cas9 can be reprogrammed to create specific double-stranded DNA breaks in the genomes of a variety of organisms, ranging from human cells to bacteria, and thus constitutes a powerful tool for genetic engineering. Here we describe recent advancements in our understanding of type II CRISPR-Cas immunity and how these studies led to revolutionary genome editing applications.

Jennifer Doudna for WEF - Q&A: Towards the end of genetic disease? (2015)
https://t.co/ycIlkFn6dT
"If the Human Genome Project mapped the genetic code, with CRISPR-Cas9 we're re-writing it."

Francis Collins on CRISPR, mRNA, COVID-19 (March 2021):
https://t.co/OkAtDzQIh6

Human bodies are not machines. But given how fast genetics is advancing, "fixing" ourselves might soon come to resemble exchanging faulty mechanical parts.

In October, two scientists shared the $3 million Breakthrough Prize in Life Sciences for inventing the CRISPR/Cas9 genome editing technology that might help us end genetic diseases, like Huntington's – by taking cells out of a patient, fixing the damaged gene, and then putting the cell back in again.

The prize was jointly awarded to Jennifer Doudna, Professor of Chemistry and Molecular and Cell Biology at the University of California, Berkeley, and Emmanuelle Charpentier, professor at the Helmholtz Centre for Infection Research at Hannover Medical School, Germany.

This is an edited transcript of an interview with Professor Doudna.

Why is it important to fix or replace genes?

> Over the last decade, there has been an explosion in genomics – we've had the human genome sequenced, and genome sequences of many other animals and plants. And this has meant that it is possible to read the code of life. We now have all the sequences of genes, including many that have mutations that lead to genetic disease.
>
> The challenge has been that up until now it has been either difficult or impossible to act on this information. There have been no good technologies to manipulate genomes, to correct the genetic mutations that lead to disease. And that's what the CRISPR/Cas9 technology does – it allows specific changes to be made in the DNA of cells and organisms to enable the correction of mutations that could otherwise lead to disease.
>
> You can think about it like a computer code. The DNA of the cell is analogous to the code that programmes a computer. Imagine that you try to run the code, and there's an error in it – then the computer doesn't run very well. It's the same in the cell of an organism. If there's a mutation in the DNA, it affects the cell's ability to grow and function normally. This results in genetic diseases like Cystic Fibrosis, Huntington's Disease and Muscular Dystrophy.
>
> CRISPR/Cas9 will have a profound impact on the development of therapeutic drugs to treat genetic diseases because it is a precision tool that allows us to study how drugs affect cells.

https://t.co/yHyVBTUkxd

> Development of CRISPR as an Antiviral Strategy to Combat SARS-CoV-2 and Influenza (November 2020):https://t.co/8pmJPgjsdJ
>
> CRISPR/Cas13: A potential therapeutic option of COVID-19 (November 2020):https://t.co/SGCS2PPBPi pic.twitter.com/fta4wWXyIq
>
> — Very 0wn (@Very_0wn) January 10, 2022

https://t.co/amGqnwA4eE

> Francis Collins, NIH:
> Researchers Publish Encouraging Early Data on COVID-19 Injections (July 2020):https://t.co/UvQVjas15M
>
> CRISPR-Based Therapy Could One Day Foil COVID-19 (March 2021):https://t.co/TMumGNrE95

"CRISPR is the workhorse; mRNA encodes it."

It's a good thing... pic.twitter.com/RXYm4nyFK7

— Very 0wn (@Very_0wn) January 10, 2022

Boston University - CRISPR Ethics: Moral Considerations for Applications of a Powerful Tool (2018): https://t.co/MuOHlQ9Hpt

Table 1. Risk-benefit considerations in CRISPR technology

	Benefit(s)	Risk(s)/Harm(s)
Basic and pre-clinical research	• New model organisms and cell lines • Increased gene-editing efficiency • High-throughput screens • Novel drug targets • Access to totipotent cells • Identification of novel signaling, regulatory, and developmental pathways • Development of novel gene-editing approaches (base editing and RNA targeting) • Knowledge advancement	• Experimentation involving human embryos is controversial and illegal in some countries • Potential for privacy and confidentiality breaches
Translational and clinical medicine	• Immunotherapy • Organoids • Novel drug targets • Artificial intelligence • Modification of pathological genes • Novel therapeutics and fertility applications • Procreative liberty • Ability to "fix" single base changes • Knowledge advancement • Potential for equitable access	• Serious injury, disability, and/or death to research participant(s) and/or offspring • Blurry distinction between therapeutic and enhancement applications, leading to potential subtle or obvious exacerbation of inequalities • Misapplications • Eugenics • Potential for inequitable access and exacerbation of inequalities
Non-therapeutic applications	• Enhancement to augment select faulty or normal human characteristics • Fortification of crops and livestock • Successful control of pests, invasive species, and reservoirs (gene drives) • Disease/infection control (e.g., malaria, dengue fever, Lyme and Chagas disease, schistosomiasis) • Ecosystem alteration to protect endangered species (gene drives) • Safety • Crop cultivation • Knowledge advancement	• Eugenics • Exacerbation of racism and inequality • Theoretical risk for damage to ecosystems • Theoretical risk of misuse
Access to CRISPR technology	• Inexpensive (technology itself) • Widely available • Profit, economic growth • Innovation	• Price gouging • Prohibitively expensive applications
Regulations for clinical research involving human subjects	• Established framework in some countries to manage research risks • Legal mechanisms for redress already exist, depending on location	• Lack of appropriate supervisory infrastructure, oversight, and/or regulatory framework in many nations • Unclear how to supervise the research even in some countries with regulatory oversight • Over-regulation might hinder progress
National and international regulations, law, and policy	• Prevention against misuses of technology • Safeguard against risky, potentially harmful conditions	• Potential to encroach on individual, scientific, and societal autonomy • Limit discovery and progress • Difficult enforcement • Lack of uniformity may create inconsistencies in applications of laws/regulations

https://t.co/WWZ59Udcyl

> Leana Wen - CNN\u2019s Medical Propagandist - Has History With CCP and Eugenics Programs:https://t.co/ndUw84omZk
>
> "Served as a consultant with China Medical Board, a Rockefeller-funded venture in China". https://t.co/VRKhEakObc pic.twitter.com/n3hZZ2XuYX
>
> — Very 0wn (@Very_0wn) January 13, 2022

The Bid, BlackRock podcast - Moderna and CRISPR Therapeutics on fighting Covid-19 (April 1, 2021): https://t.co/CNXcuFSsTh

What a joke…

Moderna and CRISPR Therapeutics on fighting Covid-19

The Bid

Investing

The Covid-19 pandemic has put genomics and immunology front and center. On the first episode of a five-part mini-series on megatrends, CEO of Moderna Stéphane Bancel and CEO of CRISPR Therapeutics Dr. Samarth Kulkarni share their perspectives as companies on the frontlines. First, Stéphane shares how Moderna innovated to produce a Covid-19 vaccine in record time and the potential of mRNA technology. Then, Samarth discusses how the medical breakthroughs of CRISPR technology will impact other medical crises like cancer and sickle cell disease in years to come.

Listen on Apple Podcasts ↗

PLAY 45 min

More Episodes

NIH, EcoHealth Alliance, Peter Daszak, U.S. Department of Defense (DARPA) - Understanding the Risk of Bat Coronavirus Emergence (2014-2019):
- https://t.co/cz53OCfZho
- Scientific Research - "Combating Weapons of Mass Destruction":
https://t.co/GBmnP3DlEc

Understanding the Risk of Bat Coronavirus Emergence

Project Number	Contact PI/Project Leader	Awardee Organization
2R01AI110964-06	DASZAK, PETER	ECOHEALTH ALLIANCE, INC.

Description

Abstract Text

Project Summary: Understanding the Risk of Bat Coronavirus Emergence Novel zoonotic, bat-origin CoVs are a significant threat to global health and food security, as the cause of SARS in China in 2002, the ongoing outbreak of MERS, and of a newly emerged Swine Acute Diarrhea Syndrome in China. In a previous R01 we found that bats in southern China harbor an extraordinary diversity of SARSr-CoVs, some of which can use human ACE2 to enter cells, infect humanized mouse models causing SARS-like illness, and evade available therapies or vaccines. We found that people living close to bat habitats are the primary risk groups for spillover, that at one site diverse SARSr-CoVs exist that contain every genetic element of the SARS-CoV genome, and identified serological evidence of human exposure among people living nearby. These findings have led to 18 published peer-reviewed papers, including two papers in Nature, and a review in Cell. Yet salient questions remain on the origin, diversity, capacity to cause illness, and risk of spillover of these viruses. In this R01 renewal we will address these issues through 3 specific aims: Aim 1. Characterize the diversity and distribution of high spillover-risk SARSr-CoVs in bats in southern China. We will use phylogeographic and viral discovery curve analyses to target additional bat sample collection and molecular CoV screening to fill in gaps in our previous sampling and fully characterize natural SARSr-CoV diversity in southern China. We will sequence receptor binding domains (spike proteins) to identify viruses with the highest potential for spillover which we will include in our experimental investigations (Aim 3). Aim 2. Community, and clinic-based syndromic, surveillance to capture SARSr-CoV spillover, routes of exposure and potential public health consequences. We will conduct biological-behavioral surveillance in high-risk populations, with known bat contact, in community and clinical settings to 1) identify risk factors for serological and PCR evidence of bat SARSr-CoVs; & 2) assess possible health effects of SARSr-CoVs infection in people. We will analyze bat-CoV serology against human-wildlife contact and exposure data to quantify risk factors and health impacts of SARSr-CoV spillover. Aim 3. In vitro and in vivo characterization of SARSr-CoV spillover risk, coupled with spatial and phylogenetic analyses to identify the regions and viruses of public health concern. We will use S protein sequence data, infectious clone technology, in vitro and in vivo infection experiments and analysis of receptor binding to test the hypothesis that % divergence thresholds in S protein sequences predict spillover potential. We will combine these data with bat host distribution, viral diversity and phylogeny, human survey of risk behaviors and illness, and serology to identify SARSr-CoV spillover risk hotspots across southern China. Together these data and analyses will be critical for the future development of public health interventions and enhanced surveillance to prevent the re-emergence of SARS or the emergence of a novel SARSr-CoV.

Public Health Relevance Statement

Program Director/Principal Investigator: Daszak, Peter Renewal: Understanding the Risk of Bat Coronavirus Emergence Project Narrative Most emerging human viruses come from wildlife, and these represent a significant threat to public health and biosecurity in the US and globally, as was demonstrated by the SARS coronavirus pandemic of 2002-03. This project seeks to understand what factors allow coronaviruses, including close relatives to SARS, to evolve and jump into the human population by studying viral diversity in their animal reservoirs (bats), surveying people that live in high-risk communities in China for evidence of bat-coronavirus infection, and conducting laboratory experiments to analyze and predict which newly-discovered viruses pose the greatest threat to human health.

https://t.co/iSR5TAOP03

> Staying in denial leads to more Pathogen X to come.
>
> DARPA: "COVID-19 is just the latest Disease X. There're will be more of them and DARPA's extensive and continuously growing platform of pandemic-stopping technologies will be ready to use in humanity's defense." https://t.co/DgGoC8KKye pic.twitter.com/8QPen0YCvO

— Very 0wn (@Very_0wn) January 14, 2022

https://t.co/0iiB82IH3D

Bill Gates, Shattuck Lecture Innovation for Pandemics, April 2018:https://t.co/0I2uTnMggh
"If we can learn how to use RNA or DNA gene delivery effectively, we may not need to make the antibodies at all."

Did you get any of that? pic.twitter.com/aCPfBJqO34

— Very 0wn (@Very_0wn) November 6, 2021

DARPA invests $100m in gene-drive technology - new gene-editing technology, which many people fear could lead to deliberate and unintended damage on a huge scale (2017):
https://t.co/MORcxOombn

Harvard, Wyss Institute - CRISPR-Cas9: Gene-drive:
https://t.co/7daNFMZjBo

By E&T editorial staff

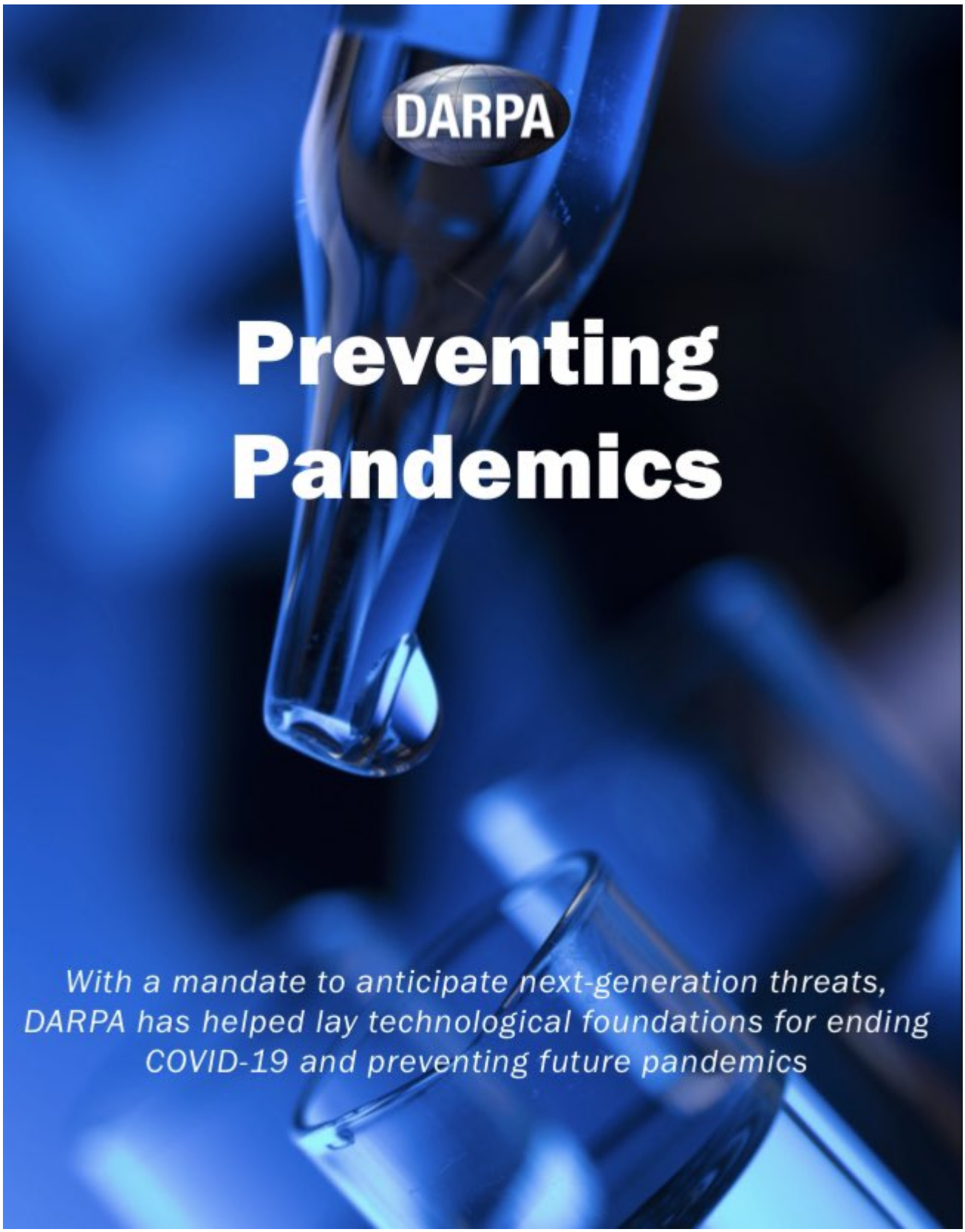
Published Thursday, December 7, 2017

The US Defense Advanced Research Projects Agency (DARPA) is investing $100 million in a new gene-editing technology, which many people fear could lead to deliberate and unintended damage on a huge scale.

Gene-drive technology uses CRISPR gene-editing to favour particular genes. This causes them to appear more frequently in offspring and, consequently, in the future population of that species. Gene-drive technology has many positive applications, such as to genetically engineer disease-carrying insects or invasive species – such as stoats in New Zealand – to impair their reproductive capabilities and cut their populations.

DARPA - Preventing Pandemics (March 2021)
https://t.co/0CSBJFgv7F

Prevending Pandemics Platform (P3):
https://t.co/Pa88DKGmzh

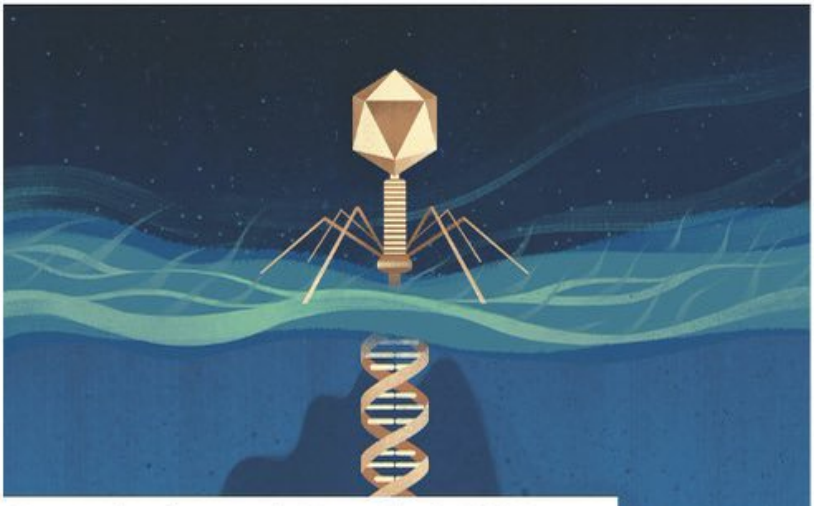


The Rockefeller University (March 2019):
https://t.co/F6QUXpPK4K

“If a phage has a single point mutation in its target sequence, then usually the virus is invisible to Cas and the infection will succeed,” says Marraffini. “But with CRISPR-Cas13 we didn’t see any escaper mutants.”

To curb infection, bacteria direct their defenses against themselves

May 30, 2019



Bacteriophages inject their DNA into bacteria, typically resulting in the bacterium's demise. CRISPR-Cas systems defend bacteria against these attacks. Illustration by Jasu Hu.

Sometimes, the best defense against hostile invaders is a good, long nap. Or at least, that strategy seems to work for bacteria.

In a new study, described in *Nature*, Rockefeller scientists showed that microbes under viral attack turn their defenses not only on their enemies, but also on themselves. This drastic measure, the researchers found, doesn't kill the bacteria, but rather sends them into a dormant state that prevents the infection from spreading.

Application of CRISPR/Cas9-Based Gene Editing in HIV-1/AIDS Therapy (2019):
https://t.co/OpFCn3uH1O
The safety and delivery efficiency of Cas9 also need consideration, since long term expression of Cas9/sgRNA may induce non-specific injury to the host genome and immune response.

Review

Application of CRISPR/Cas9-Based Gene Editing in HIV-1/AIDS Therapy

Qiaoqiao Xiao et al. Front Cell Infect Microbiol. 2019.

Free PMC article

Abstract

Despite the fact that great efforts have been made in the prevention and therapy of HIV-1 infection, HIV-1/AIDS remains a major threat to global human health. Highly active antiretroviral therapy (HAART) can suppress virus replication, but it cannot eradicate latent viral reservoirs in HIV-1/AIDS patients. Recently, the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated nuclease 9 (Cas9) system has been engineered as an effective gene-editing technology with the potential to treat HIV-1/AIDS. It can be used to target cellular co-factors or HIV-1 genome to reduce HIV-1 infection and clear the provirus, as well as to induce transcriptional activation of latent virus in latent viral reservoirs for elimination. This versatile gene editing technology has been successfully applied to HIV-1/AIDS prevention and reduction in human cells and animal models. Here, we update the rapid progress of CRISPR/Cas9-based HIV-1/AIDS therapy research in recent years and discuss the limitations and future perspectives of its application.

Keywords: CRISPR/Cas9; HIV-1/AIDS; gene editing; host factors; latent viral reservoirs.

CRISPR/Cas9-Derived Mutations Both Inhibit HIV-1 Replication and Accelerate Viral Escape (2016): https://t.co/y2GreH3EB2

CRISPR/Cas9-Derived Mutations Both Inhibit HIV-1 Replication and Accelerate Viral Escape

Zhen Wang et al. Cell Rep. 2016.

Free article

Abstract

Cas9 cleaves specific DNA sequences with the assistance of a programmable single guide RNA (sgRNA). Repairing this broken DNA by the cell's error-prone non-homologous end joining (NHEJ) machinery leads to insertions and deletions (indels) that often impair DNA function. Using HIV-1, we have now demonstrated that many of these indels are indeed lethal for the virus, but that others lead to the emergence of replication competent viruses that are resistant to Cas9/sgRNA. This unexpected contribution of Cas9 to the development of viral resistance is facilitated by some indels that are not deleterious for viral replication, but that are refractory to recognition by the same sgRNA as a result of changing the target DNA sequences. This observation illustrates two opposite outcomes of Cas9/sgRNA action, i.e., inactivation of HIV-1 and acceleration of viral escape, thereby potentially limiting the use of Cas9/sgRNA in HIV-1 therapy.

DARPA Microphysiological Systems:
https://t.co/fNg9lUxfLc

U.S. Department of Defense:
https://t.co/oefauN4lz7
“The interactions that candidate ■ and ■ have with these mimics will accurately predict the safety and effectiveness the countermeasures would have if given to ■.” https://t.co/io8sA205fD

DARPA

MICROPHYSIOLOGICAL SYSTEMS

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A Crispr-Cas9 System Designed to Introduce Point Mutations into the Human ACE2 Gene to Weaken the Interaction of the ACE2 Receptor with the SARS-CoV-2 S Protein (May 2020):
https://t.co/JuarmcFR9u
https://t.co/HenCBrrRxD

preprints.org > life sciences > molecular biology > doi: 10.20944/preprints202005.0134.v1

Preprint Article Version 1 Preserved in Portico This version is not peer-reviewed

A Crispr-Cas9 System Designed to Introduce Point Mutations into the Human *ACE2* Gene to Weaken the Interaction of the ACE2 Receptor with the SARS-CoV-2 S Protein

Pedro Tanaka , Jadson Santos , Ernna Oliveira , Natane Miglioli , Amanda Assis , Ana Monteleone-Cassiano , Vitor Ribeiro , Max Duarte , Mayara Machado , Romario Mascarenhas , Alex Souza , Leticia Brito , Leticia Oliveira , Eduardo Donadi , Geraldo Passos *

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Abstract

The human angiotensin-converting enzyme 2 (ACE2) has a crucial role on blood pressure control; however, ACE2 is also the primary SARS-CoV-2 (S domain) virus receptor. Inhibiting or even reducing the expression of the native ACE2 might diminish the viral entry into the cells, but may cause a failure of ACE2 biological activity, primarily in patients with comorbidities, including diabetes mellitus or hypertension. Since the ACE2 catalytic site and the SARS-Cov-2 receptor are distinct, we designed a Crispr-Cas9 model system, predicting the respective sequences for a guide RNA (gRNA) and a single-stranded oligo dideoxy nucleotide (ssODN), to introduce point mutations into the exon 1 of the human *ACE2* gene, which encodes the alpha-helix, implicated on the binding of the SARS-CoV-2 envelope S protein. Protein modeling predicted that the specific substitutions of residues Phe28, Lys31, and Tyr41 for Ala at the ACE2 alpha-helix do not significantly alter ACE2 native conformation. The analysis of the impact of these mutations on ACE2 receptor function predicted a weakening of the binding of the SARS-CoV-2 protein S. An experimental genome editing of cells based on these Crispr-Cas9 elements might reduce the SARS-CoV-2 ability to enter the epithelial cell, preserving the biological activity of ACE2 enzyme.

Supplementary and Associated Material

http://www.rge.fmrp.usp.br/passos/off_targets_gRNA_ACE2: Human ACE2 gRNA off-targets

Time - mRNA Technology Gave Us the First COVID-19 Injections (January 2021):
https://t.co/DELjdJGG1x
“CRISPR is also being used in the war against COVID. Doudna and others have created RNA-guided enzymes that can directly detect SARS-CoV-2 and eventually could be used to destroy”

NIH, Francis Collins, Dr. Fauci (March 2020):

'These are dramatic times for NIH research. Today, I focused on gene-based therapies. The CRISPR provides "find and

replace" function for DNA.'

What are genome editing and CRISPR-Cas9?
https://t.co/vLihnXKvYn

https://t.co/psJ8lqzun4

NIH @NIH · 04 Mar 20

.@NIHDirector on the Hill: Latest development in gene-based treatments, the CRISPR, promises to boost that number. #CRISPR provides a precise “find & replace” function for DNA, allowing cells to be reprogrammed to correct disease-causing misspellings ghr.nlm.nih.gov/primer/genomic... #NIH

medlineplus.gov

What are genome editing and CRISPR-Cas9?: MedlinePlus G...

1 3 6

Congressional Research Service - Advanced Gene Editing: CRISPR-Cas9 (December 2018): https://t.co/27weYrBOSC

Summary

Scientists have long sought the ability to control and modify DNA—the code of life. A gene editing technology known as CRISPR-Cas9 offers the potential for substantial improvement over other gene editing technologies in that it is simple to use and inexpensive and has a relatively high degree of precision and efficiency. These characteristics have led many in the scientific and business communities to assert that CRISPR-Cas9 will lead to groundbreaking advances in many fields, including agriculture, energy, ecosystem conservation, and the investigation, prevention, and treatment of diseases.

Over the next 5 to 10 years, the National Academy of Sciences projects a rapid increase in the scale, scope, complexity, and development rate of biotechnology products, many enabled by CRISPR-Cas9. Concomitant with the promise of potential benefits, such advances may pose new risks and raise ethical concerns. For example, a Chinese researcher recently claimed that he had created the first genetically engineered human babies. According to the researcher, he used CRISPR-Cas9 to disable a gene that will make it harder for the twin girls, who were born in November 2018, to contract human immunodeficiency virus (HIV). The as yet unsubstantiated claim has sparked outrage and ethical debates by the international scientific community and others. Prior use of CRISPR-Cas9 gene editing in human embryos was generally limited to nonviable embryos, in part, to address ethical concerns such as the fact that the genetic change would affect not only the immediate patient, but also future generations who would inherit the change.

Additionally, CRISPR-related approaches (gene drives) are being considered to reduce or eliminate the mosquito that serves as the primary vector for the transmission of Zika or malaria, thereby improving public health. Some scientists and environmental groups have raised ethical questions and expressed concerns about the unintended ecological consequences of eliminating a species or introducing a genetically modified organism into an open environment.

Some experts assert that the current system for regulating biotechnology products—the Coordinated Framework for the Regulation of Biotechnology—may be inadequate, with the potential to leave gaps in oversight. Regulatory gaps may lead to increased uncertainty that could affect the development of future biotechnology products or a loss of public confidence in the ability of regulators to ensure that such products are safe.

In the 116th Congress, policymakers may want to examine the potential benefits and risks associated with the use of CRISPR-Cas9 gene editing, including the ethical, social, and legal implications of CRISPR-related biotechnology products. Congress also may have a role to play with respect to regulation, research and development, and economic competitiveness associated with CRISPR-Cas9 gene editing and future biotechnology products.

CRISPR-Cas9 to knock down microRNA in vitro and in vivo (2016):
https://t.co/PqCvspzGTK
"CRISPR-Cas9 can robustly and specifically reduce the expression of these microRNAs up to 96%"
|
"the dysregulation of microRNAs has been associated with cancer"
https://t.co/y7QzTrZTO0

Open Access | Published: 29 February 2016

CRISPR/cas9, a novel genomic tool to knock down microRNA *in vitro* and *in vivo*

Hong Chang, Bin Yi, Ruixia Ma, Xiaoguo Zhang, Hongyou Zhao & Yaguang Xi

Scientific Reports **6**, Article number: 22312 (2016) | Cite this article

28k Accesses | **120** Citations | **12** Altmetric | Metrics

Abstract

MicroRNAs are small and non-coding RNA molecules with the master role in regulation of gene expression at post-transcriptional/translational levels. Many methods have been developed for microRNA loss-of-function study, such as antisense inhibitors and sponges; however, the robustness, specificity, and stability of these traditional strategies are not highly satisfied. CRISPR/cas9 system is emerging as a novel genome editing tool in biology/medicine research, but its indication in microRNA research has not been studied exclusively. In this study, we clone CRISPR/cas9 constructs with single-guide RNAs specifically targeting biogenesis processing sites of selected microRNAs; and we find that CRISPR/cas9 can robustly and specifically reduce the expression of these microRNAs up to 96%. CRISPR/cas9 also shows an exclusive benefit in control of crossing off-target effect on microRNAs in the same family or with highly conserved sequences. More significantly, for the first time, we demonstrate the long term stability of microRNA knockdown phenotype by CRISPR/cas9 in both *in vitro* and *in vivo* models.

U.S. Military Preps for Gene Drives Run Amok (2016):
https://t.co/KgS7tqD6WR

Bill Gates - We Need to Embrace CRISPR Gene-Editing for Global Development (2018):
https://t.co/ihJOGAMt30

Bill Gates - How CRISPR could save lives and end diseases (2021):

https://t.co/okwuhoAo39

Over the next four years a new program in the Pentagon's Defense Advanced Research Projects Agency (DARPA) plans to cultivate, among other things, a kind of cleanup crew for engineered genes deemed harmful to or undesirable in an ecosystem. The initiative, called Safe Genes, comes at a time when so-called "gene drive" systems, which override the standard rules of gene inheritance and natural selection, are raising hopes among some scientists that the technology could alter or suppress populations of disease-carrying insects or other pests in as few as 20 generations.

The Bill and Melinda Gates Foundation sees so much promise in gene drive technology that it plans to double spending on its Target Malaria initiative, which aims to create systems for driving genes in two species of malaria mosquitoes, to $70 million. Yet without careful precautions, a gene drive released into the wild could spread or change in unexpected ways. Kevin Esvelt, head of the Sculpting Evolution lab at MIT Media Lab, which is applying for Safe Genes funding in collaboration with eight other research groups, predicts that eventually, perhaps around 15 years from now, an accident will allow a drive with potential to spread globally to escape laboratory controls. "It's not going to be bioterror," he says, "it's going to be 'bioerror.'"

DARPA itself has been one of the largest public funders of synthetic biology research in the U.S. in recent years, upping its spending on synthetic biology projects to more than $100 million in 2014 from nothing in 2010, according to one analysis. The agency announced its Safe Genes program in September 2016 and plans to award funding to multiple research teams by the first half of 2017. "If we're going to be really bullish about genome engineering," says DARPA program manager Renee Wegrzyn, "we need to be just as aggressive with tools to reverse those changes."

Normally, a parent organism with a given trait passes that genetic code to offspring about half the time. Recent advances combining the gene-editing tool CRISPR–Cas9 (for, clustered, regularly interspaced, short palindromic repeats with a guiding enzyme called Cas9) are now making it easier for scientists to modify a genome such that nearly all offspring inherit the desired trait.

The Rockefeller University Hosts Panel On Human Genome Editing (2017):
https://t.co/Rct1VzeKdm

Dr. Matthew Liao, a bioethicist, suggests CRISPR for:
- cat eyes
- pharmacological induction of empathy
- hobbit people
- cognitive enhancement
- meat allergies
https://t.co/bddjG1a2W9

The Rockefeller University Hosts Panel On Human Genome Editing

By Julianna LeMieux — February 14, 2017

www.letsintern.com

Human genome editing, like self-driving cars or drone delivery, is becoming a part of our everyday reality faster than we realize it.

A panel discussion held at The Rockefeller University entitled "The Future of Gene Editing: A multi-disciplinary panel discussion" brought together four experts who tackle the challenges of human gene-editing from different approaches and perspectives, based on their individual focuses and specialties. Why does this particular area of science need so much conversation?

Related articles

FDA Call For Comments On Genetically Edited Food

Will COVID End the Anti-GMO Movement?

How 'Victor/Victoria' Mosquitoes Can Help Cure Zika

CRISPR May Redefine What it Means To Be GMO

Ethicists: We Need More Flexible Tools for Evaluating Gene-Edited Food

There are significant concerns, to be sure, especially about unintended consequences. People are particularly nervous about gene drive technology and the release of altered species into the environment. Fears include these altered species entering the food chain, causing the extinction of their or another species and the creating of organisms that we never thought possible, if mutations were to occur, such as super bugs.

Somewhere in the middle of these extremes lies an incredibly difficult, emotional, human conversation that needs to draw lines in new territory where we are not comfortable and have had no practice finding boundaries.

Jamie Metzl (Senior Fellow, Atlantic Council) introduced the subject with the perspective that gene editing is the single most important topic that should be discussed at this moment in time. Why? Because, as Metzl said, "this is the time when our species took control of our evolutionary process." Moreover, there is no clear answer as to where lines should be drawn between scientific progress and going too far.

One option would be to throw up our hands and let people do what they want - why restrict science? But, the technology behind CRISPR can be done by almost anyone with a pipette, so, we must define the rules of engagement. But, stifling all progress because we are scared of the unknown is also not the best answer.

2020 Nobel Prize for Chemistry awarded for the development of a method for genome editing: https://t.co/jF2EBz9OcU
Using CRISPR-Cas9 genetic scissors, researchers can change the DNA of animals, plants and microorganisms with extremely high precision - to rewrite the code of life.

Genetic scissors: a tool for rewriting the code of life

Emmanuelle Charpentier and Jennifer A. Doudna have discovered one of gene technology's sharpest tools: the CRISPR/Cas9 genetic scissors. Using these, researchers can change the DNA of animals, plants and microorganisms with extremely high precision. This technology has had a revolutionary impact on the life sciences, is contributing to new cancer therapies and may make the dream of curing inherited diseases come true.

Researchers need to modify genes in cells if they are to find out about life's inner workings. This used to be time-consuming, difficult and sometimes impossible work. Using the CRISPR/Cas9 genetic scissors, it is now possible to change the code of life over the course of a few weeks.

"There is enormous power in this genetic tool, which affects us all. It has not only revolutionised basic science, but also resulted in innovative crops and will lead to ground-breaking new medical treatments," says Claes Gustafsson, chair of the Nobel Committee for Chemistry.

As so often in science, the discovery of these genetic scissors was unexpected. During Emmanuelle Charpentier's studies of *Streptococcus pyogenes*, one of the bacteria that cause the most harm to humanity, she discovered a previously unknown molecule, *tracrRNA*. Her work showed that tracrRNA is part of bacteria's ancient immune system, *CRISPR/Cas*, that disarms viruses by cleaving their DNA.

Charpentier published her discovery in 2011. The same year, she initiated a collaboration with Jennifer Doudna, an experienced biochemist with vast knowledge of RNA. Together, they succeeded in recreating the bacteria's genetic scissors in a test tube and simplifying the scissors' molecular components so they were easier to use.

In an epoch-making experiment, they then reprogrammed the genetic scissors. In their natural form, the scissors recognise DNA from viruses, but Charpentier and Doudna proved that they could be controlled so that they can cut any DNA molecule at a predetermined site. Where the DNA is cut it is then easy to rewrite the code of life.

Recent Advances in CRISPR/Cas9 Delivery Strategies (2020):
https://t.co/WuGrKXlowK
'The CRISPR/Cas9 system can be delivered in the format of DNA (“all-in-one” plasmid), mRNA (Cas9 mRNA and sgRNA), or protein (RNP).'
https://t.co/ujox6aqp9f

Biomolecules. 2020 Jun; 10(6): 839.
Published online 2020 May 30. doi: 10.3390/biom10060839
PMCID: PMC7356196
PMID: 32486234

Recent Advances in CRISPR/Cas9 Delivery Strategies

Bon Ham Yip

Abstract

Go to:

The clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system has revolutionized the field of gene editing. Continuous efforts in developing this technology have enabled efficient in vitro, ex vivo, and in vivo gene editing through a variety of delivery strategies. Viral vectors are commonly used in in vitro, ex vivo, and in vivo delivery systems, but they can cause insertional mutagenesis, have limited cloning capacity, and/or elicit immunologic responses. Physical delivery methods are largely restricted to in vitro and ex vivo systems, whereas chemical delivery methods require extensive optimization to improve their efficiency for in vivo gene editing. Achieving a safe and efficient in vivo delivery system for CRISPR/Cas9 remains the most challenging aspect of gene editing. Recently, extracellular vesicle-based systems were reported in various studies to deliver Cas9 in vitro and in vivo. In comparison with other methods, extracellular vesicles offer a safe, transient, and cost-effective yet efficient platform for delivery, indicating their potential for Cas9 delivery in clinical trials. In this review, we first discuss the pros and cons of different Cas9 delivery strategies. We then specifically review the development of extracellular vesicle-mediated gene editing and highlight the strengths and weaknesses of this technology.

Keywords: CRISPR/Cas9, gene editing, delivery, extracellular vesicles, virus-like particles

Modeling human disease in rodents by CRISPR/Cas9 genome editing (2017):
https://t.co/MKzpIOHrUX
|
Self-destructing mosquitoes and sterilized rodents: the promise of gene drives (2019):
https://t.co/9pSCM9xnrB
https://t.co/LLTajEN2qP

Mamm Genome. 2017; 28(7): 291–301.
Published online 2017 Jul 4. doi: 10.1007/s00335-017-9703-x
PMCID: PMC5569124
PMID: 28677007

Modeling human disease in rodents by CRISPR/Cas9 genome editing

Marie-Christine Birling,[1] Yann Herault,[1,2,3,4,5] and Guillaume Pavlovic[1]

Author information ▸ Article notes ▸ Copyright and License information Disclaimer

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Marie-Christine Birling, Email: birlingm@igbmc.fr.
Corresponding author.

Abstract

Go to:

Modeling human disease has proven to be a challenge for the scientific community. For years, generating an animal model was complicated and restricted to very few species. With the rise of CRISPR/Cas9, it is now possible to generate more or less any animal model. In this review, we will show how this technology is and will change our way to obtain relevant disease animal models and how it should impact human health.

Introduction

Go to:

Genome editing and especially the easy and accessible CRISPR/Cas9 technology have open new opportunities in modeling human diseases. Genome-Wide Association Studies (GWAS) with specific point mutations, mutation in coding and non-coding genes, copy number variants (CNVs), and regulatory mutations are now feasible in more or less any genetic background and any species. In this review, we will focus on the latest advancements in the development of disease model in rodents. Because of their phylogenetic relatedness and physiological similarity to humans, their maintenance facility, and easy breeding in the laboratory, mice and rats are the most widely used organisms in research. Genetically engineered rodents allowed major discoveries but sometimes failed to translate to human.

Stroke. Author manuscript; available in PMC 2013 Dec 1.
Published in final edited form as:
Stroke. 2012 Dec; 43(12): 3358–3363.
Published online 2012 Nov 15. doi: 10.1161/STROKEAHA.112.667063

PMCID: PMC3529166
NIHMSID: NIHMS419940
PMID: 23160880

IMPACT OF ACE2 DEFICIENCY AND OXIDATIVE STRESS ON CEREBROVASCULAR FUNCTION WITH AGING

Ricardo A. Peña Silva, MD,[1,3] Yi Chu, PhD,[2] Jordan D. Miller, PhD,[2] Ian J. Mitchell, BSc,[2] Josef M. Penninger, MD, PhD,[4] Frank M. Faraci, PhD,[1,2] and Donald D. Heistad, MD[1,2]

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Abstract

Go to:

Background and Purpose

Angiotensin II produces oxidative stress and endothelial dysfunction in cerebral arteries, and angiotensin II type I receptors may play a role in longevity and vascular aging. Angiotensin converting enzyme type 2 (ACE2) converts angiotensin II to angiotensin (1–7) and thus may protect against effects of angiotensin II. We hypothesized that ACE2 deficiency increases oxidative stress and endothelial dysfunction in cerebral arteries, and examined the role of ACE2 in age-related cerebrovascular dysfunction.

Methods

Endothelial function, expression of angiotensin system components, NADPH oxidase subunits, and proinflammatory cytokines were examined in cerebral arteries from adult [12 mo old] and old [24 mo old] ACE2 knockout (KO) and wild type (WT) mice. The superoxide scavenger tempol was used to examine the role of oxidative stress on endothelial function.

Results

Vasodilatation to acetylcholine was impaired in adult ACE2 KO [24±6% (mean +/− SE)] compared to WT mice [52±7%, $p<0.05$]. In old mice, vasodilatation to acetylcholine was impaired in WT mice [29±6%] and severely impaired in ACE2 KO mice [7±5%]. Tempol improved endothelial function in adult and old ACE2 KO and WT mice. Aging increased mRNA for TNFα in WT mice, and significantly increased mRNA levels of Nox2, $p47^{phox}$, and Rcan1 in both ACE2 KO and WT mice. mRNA levels of angiotensin system components did not change during aging.

Conclusions

ACE2 deficiency impaired endothelial function in cerebral arteries from adult mice and augmented endothelial dysfunction during aging. Oxidative stress plays a critical role in cerebrovascular dysfunction induced by ACE2 deficiency and aging.

Keywords: Endothelium, angiotensin converting enzyme 2, aging, cerebral arteries, oxidative stress

mRNA-based therapeutics - a new class of drugs (2014):
https://t.co/r3HY2N37jE

Development of CRISPR-Cas9 mRNA for gene-editing (2013):
https://t.co/vFJfkbwCLT

"zebrafish has the same major organs and tissues as humans; ability to repair heart muscle"
https://t.co/9eqZpz1Rdy

Published: 19 September 2014

mRNA-based therapeutics – developing a new class of drugs

Ugur Sahin, Katalin Karikó & Özlem Türeci

Nature Reviews Drug Discovery **13**, 759–780 (2014) | Cite this article

Key Points

- Messenger RNA (mRNA) is a pivotal molecule of life, involved in almost all aspects of cell biology.
- As the subject of basic and applied research for more than 5 decades, mRNA has only recently come into the focus as a potentially powerful drug class able to deliver genetic information.
- Synthetic mRNA can be engineered to resemble mature and processed mRNA molecules as they occur naturally in the cytoplasm of eukaryotic cells and to transiently deliver proteins.
- Recent advances addressed challenges inherent to this drug class and provided the basis for a broad spectrum of applications
- Besides cancer immunotherapies and infectious disease vaccines novel approaches such as *in vivo* delivery of mRNA to replace or supplement proteins, mRNA-based induction of pluripotent stem cells, or mRNA-assisted delivery of designer nucleases for genome engineering rapidly emerged and entered into pharmaceutical development.
- This Review gives a comprehensive overview of the current state of mRNA drug technologies, their applications and crucial aspects relevant to mRNA based drug discovery and development.

Matthew Liao - Selecting Children: The Ethics of Reproductive Genetic Engineering (2008): https://t.co/VvOTRqyDGa

Compulsory moral bioenhancement should be covert (August 2018):
https://t.co/giU0hN0uWR

H/t: @JesseMatchey, @maraki378
https://t.co/vBCv2eTjMq

› Bioethics. 2019 Jan;33(1):112-121. doi: 10.1111/bioe.12496. Epub 2018 Aug 29.

Compulsory moral bioenhancement should be covert

Parker Crutchfield [1]

Affiliations + expand

PMID: 30157295 DOI: 10.1111/bioe.12496

Abstract

Some theorists argue that moral bioenhancement ought to be compulsory. I take this argument one step further, arguing that if moral bioenhancement ought to be compulsory, then its administration ought to be covert rather than overt. This is to say that it is morally preferable for compulsory moral bioenhancement to be administered without the recipients knowing that they are receiving the enhancement. My argument for this is that if moral bioenhancement ought to be compulsory, then its administration is a matter of public health, and for this reason should be governed by public health ethics. I argue that the covert administration of a compulsory moral bioenhancement program better conforms to public health ethics than does an overt compulsory program. In particular, a covert compulsory program promotes values such as liberty, utility, equality, and autonomy better than an overt program does. Thus, a covert compulsory moral bioenhancement program is morally preferable to an overt moral bioenhancement program.

Keywords: autonomy; harm; moral enhancement; public health ethics; public policy.

Comment in

Covert moral bioenhancement, public health, and autonomy.
Zambrano A.
Bioethics. 2019 Jul;33(6):725-728. doi: 10.1111/bioe.12567. Epub 2019 Apr 15.
PMID: 30989673

It is better to be ignorant of our moral enhancement: A reply to Zambrano.
Crutchfield P.
Bioethics. 2020 Feb;34(2):190-194. doi: 10.1111/bioe.12685. Epub 2019 Oct 22.
PMID: 31639224

George Church, Martin Nowak - Evolutionary dynamics of CRISPR-Cas9 gene drives (2016):
https://t.co/f4ITVRwUJy

Matthew Liao - Designing humans: A human rights approach (2018):
https://t.co/s14Ph02wRD
- 'Is Gene Editing Ethical? It Depends'

https://t.co/Fc1s6eBwLI

Evolutionary dynamics of CRISPR gene drives

Charleston Noble, Jason Olejarz, Kevin M. Esvelt, George M. Church, Martin A. Nowak

doi: https://doi.org/10.1101/057281

Now published in *Science Advances* doi: 10.1126/sciadv.1601964

Abstract

The alteration of wild populations has been discussed as a solution to a number of humanity's most pressing ecological and public health concerns. Enabled by the recent revolution in genome editing, CRISPR gene drives, selfish genetic elements which can spread through populations even if they confer no advantage to their host organism, are rapidly emerging as the most promising approach. But before real-world applications are considered, it is imperative to develop a clear understanding of the outcomes of drive release in nature. Toward this aim, we mathematically study the evolutionary dynamics of CRISPR gene drives. We demonstrate that the emergence of drive-resistant alleles presents a major challenge to previously reported constructs, and we show that an alternative design which selects against resistant alleles greatly improves evolutionary stability. We discuss all results in the context of CRISPR technology and provide insights which inform the engineering of practical gene drive systems.

Main Text

Gene drive systems are selfish genetic elements which bias their own inheritance and spread through populations in a super-Mendelian fashion (Fig. 1A). Such elements have been discussed as a means of contributing to the eradication of insect-borne diseases such as malaria, reversing herbicide and pesticide resistance in agriculture, and controlling destructive invasive species (*1–12*).Various examples of gene drive can be found in nature, including transposons (*13*), Medea elements (*14*, *15*), and segregation distorters (*16–19*), but for ecological engineering purposes, endonuclease gene drive systems have received the most significant attention in the literature (*1–10*, *20–22*). In general, these elements function by converting drive-heterozygotes into drive-homozygotes through a two-step process: (i) the drive construct, encoding a sequence-specific endonuclease, induces a double-strand break (DSB) at its own position on a homologous chromosome, and (ii) subsequent DSB repair by homologous recombination (HR) copies the drive into the break site. Any sequence adjacent to the endonuclease will be copied as well; if a gene is present we refer to it as 'cargo', as it is 'driven' by the endonuclease through the population.

CRISPR-Cas9: Transhumanism and Designing the Living (2018):
https://t.co/1ulbtFfvdN

The Rockefeller Foundation - Bionics, Transhumanism, and the End of Evolution (2019):
https://t.co/yYS69gR1cC

"Jeffrey Epstein was a transhumanist."

H/t: @JesseMatchey
https://t.co/5uOJc0dBag

What is the fourth industrial revolution?

A new era is beginning that builds and extends the impact of digitization in unanticipated ways Image: REUTERS/Reinhard Krause

19 Jan 2016

Nicholas Davis
Professor of Practice, Thunderbird School of Global Management and Visiting Professor in Cybersecurity, UCL Department of Science, Technology, Engineering and Public Policy

Are the technologies that surround us tools that we can identify, grasp and consciously use to improve our lives? Or are they more than that: powerful objects and enablers that influence our perception of the world, change our behaviour and affect what it means to be human?

Technologies are emerging and affecting our lives in ways that indicate we are at the beginning of a Fourth Industrial Revolution, a new era that builds and extends the impact of digitization in new and unanticipated ways. It is therefore worthwhile taking some time to consider exactly what kind of shifts we are experiencing and how we might, collectively and individually, ensure that it creates benefits for the many, rather than the few.

When were the other industrial revolutions?

The First Industrial Revolution is widely taken to be the shift from our reliance on animals, human effort and biomass as primary sources of energy to the use of fossil fuels and the mechanical power this enabled. The Second Industrial Revolution occurred between the end of the 19th century and the first two decades of the 20th century, and brought major breakthroughs in the form of electricity distribution, both wireless and wired communication, the synthesis of ammonia and new forms of power generation. The Third Industrial Revolution began in the 1950s with the development of digital systems, communication and rapid advances in computing power, which have enabled new ways of generating, processing and sharing information.

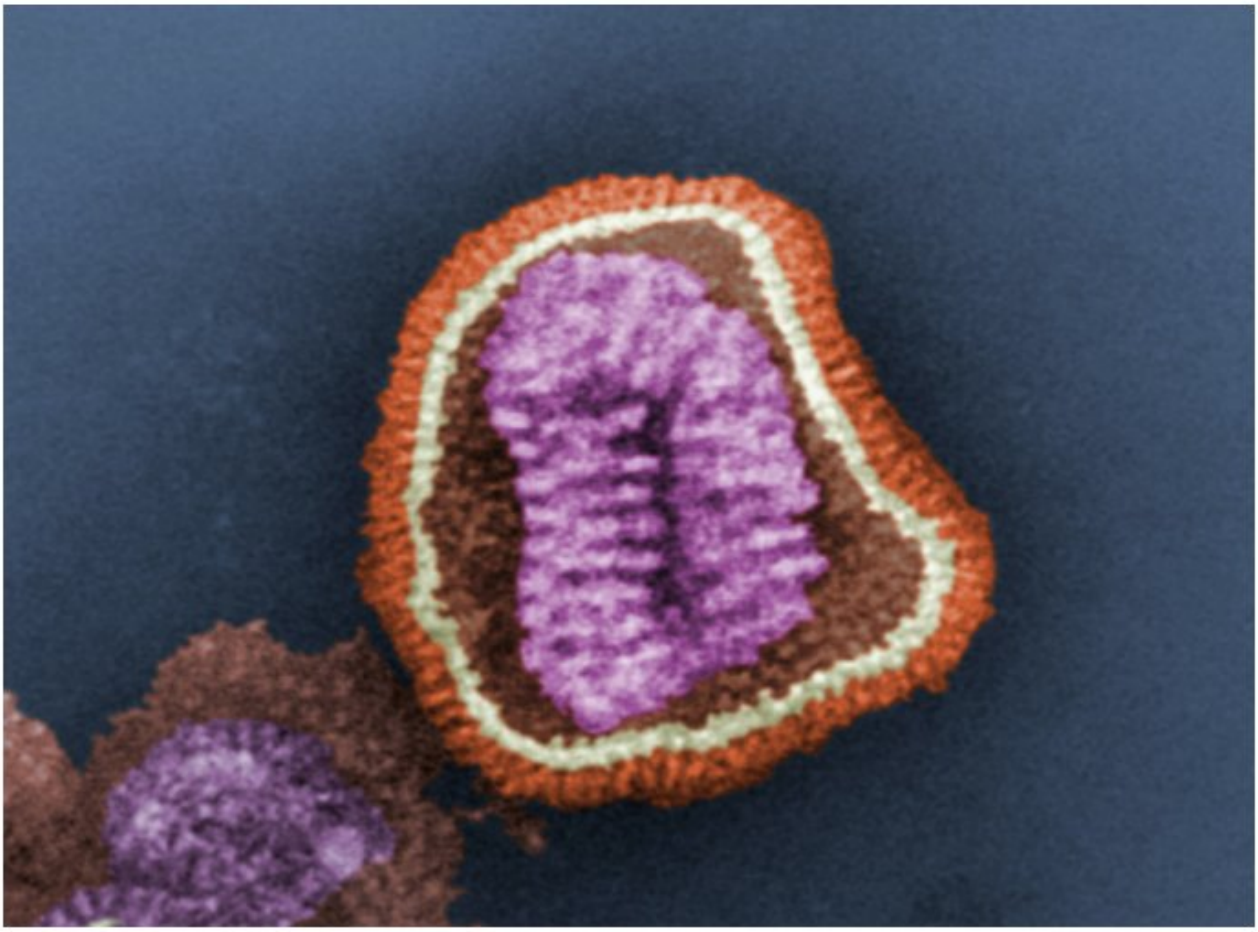
$21.9M Gene Modulation Research, Effort Targets Influenza Pandemics (June 2019):
https://t.co/CZumqc0OnO
"CDC will also partner with the team and will be funded separately by DARPA in this 4-year initiative."

Dr. Amy Jenkins - P3 & PREPARE DARPA Programs.

https://t.co/2PdksFhCER

$21.9 Million Gene Modulation Research Effort Targets Influenza Pandemics

A new $21.9 million research initiative will modulate gene expression to help protect against pandemic flu.



A digitally-colorized, negatively-stained transmission electron microscope image shows the details of an influenza virus particle. (Credit: CDC - Frederick Murphy)

Jun 28, 2019 — Atlanta, GA

A multifaceted research effort aimed at temporarily modulating gene expression using RNA-based techniques could help protect against pandemic flu by boosting lung resistance to infection, attacking the influenza virus directly, enhancing immune system response and improving the effects of existing vaccines.

Drugs developed through the up to $21.9 million effort, which is funded by the Defense Advanced Research Projects Agency (DARPA), would provide rapid response against a broad range of flu variants — and could potentially be used against other viruses in the future. The RNA-based drugs could be delivered to the lungs through a nebulizer or inhaler, which are well-established techniques.

Dr. Amy Jenkins Programs (DARPA, BTO):
- ADEPT
- INTERCEPT
- NOW
- P3
- PREPARE
- RPM
https://t.co/L5u6tTzeaY

Consultant to NIH's Somatic Cell Genome Editing Program – Phase 2 (FY23-27)
https://t.co/9LZVDiGQQg

NIH SCGE:
- https://t.co/ImLDVh5d6U
- https://t.co/5YbNvLLoNK

PROGRAMS

Autonomous Diagnostics to Enable Prevention and Therapeutics (ADEPT)

The Autonomous Diagnostics to Enable Prevention and Therapeutics (ADEPT) program supports individual troop readiness and total force health protection by developing technologies to rapidly identify and respond to threats posed by natural and engineered diseases and toxins. A subset of ADEPT technologies specifically support use by personnel with minimal medical training, delivering centralized laboratory capabilities even in the low-resource environments typical of many military operations. The program is part of a portfolio of DARPA-funded research aimed at providing options for preempting or mitigating constantly evolving infectious disease threats.

INTERfering and Co-Evolving Prevention and Therapy (INTERCEPT)

Viral pathogens pose a continuous and shifting biological threat to military readiness and national security overall in the form of infectious disease with pandemic potential. Today's limited vaccines and other antivirals are often circumvented by quickly mutating viruses that evolve to develop resistance to treatments that are carefully formulated to act only specific strains of a virus.

Nucleic acids On-demand Worldwide (NOW)

The Nucleic acids On-demand Worldwide (NOW) program aims to develop a mobile medical countermeasure (MCM) manufacturing platform for use in stabilization and humanitarian operations to rapidly produce, formulate, and package hundreds of doses of nucleic acid therapeutics (DNA and/or RNA).

U.S. Army, Pfizer - COVID-19 Pandemic, Large Scale
Injections Manufacturing Demonstration (July 2020):
https://t.co/5CyLkTLEmL
"Pfizer has self-certified that it meets the
definition of a Nontraditional Defense Contractor as defined in the Base Agreement."
https://t.co/kiIQOqOBi4

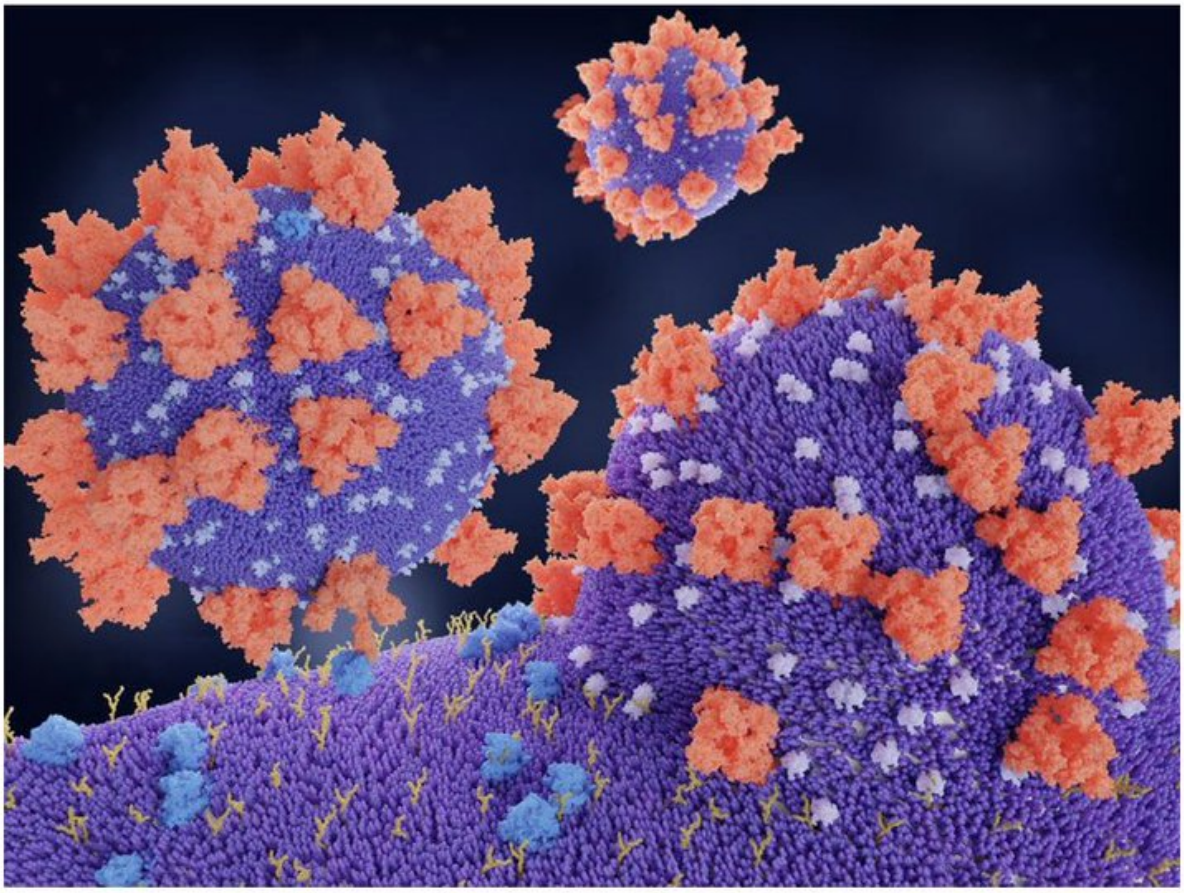
11.9 Non-Traditional Defense Contractor. Pfizer has self-certified that Pfizer meets the definition of a "Nontraditional Defense Contractor" as defined in the Base Agreement and therefore is not subject to the cost-sharing requirement referenced in Article VI of the Base Agreement.

What is the ACE2 receptor, how is it connected to coronavirus and why might it be key to treating COVID-19? (May 2020):
https://t.co/nSrQWtWOrE
"ACE2 is present in many cell types and tissues including the lungs, heart, blood vessels, kidneys, liver and gastrointestinal tract." https://t.co/POJTwbpzcl

What is the ACE2 receptor, how is it connected to coronavirus and why might it be key to treating COVID-19? The experts explain

May 14, 2020 8.04am EDT

Krishna Sriram, Paul Insel, Rohit Loomba, *University of California San Diego*

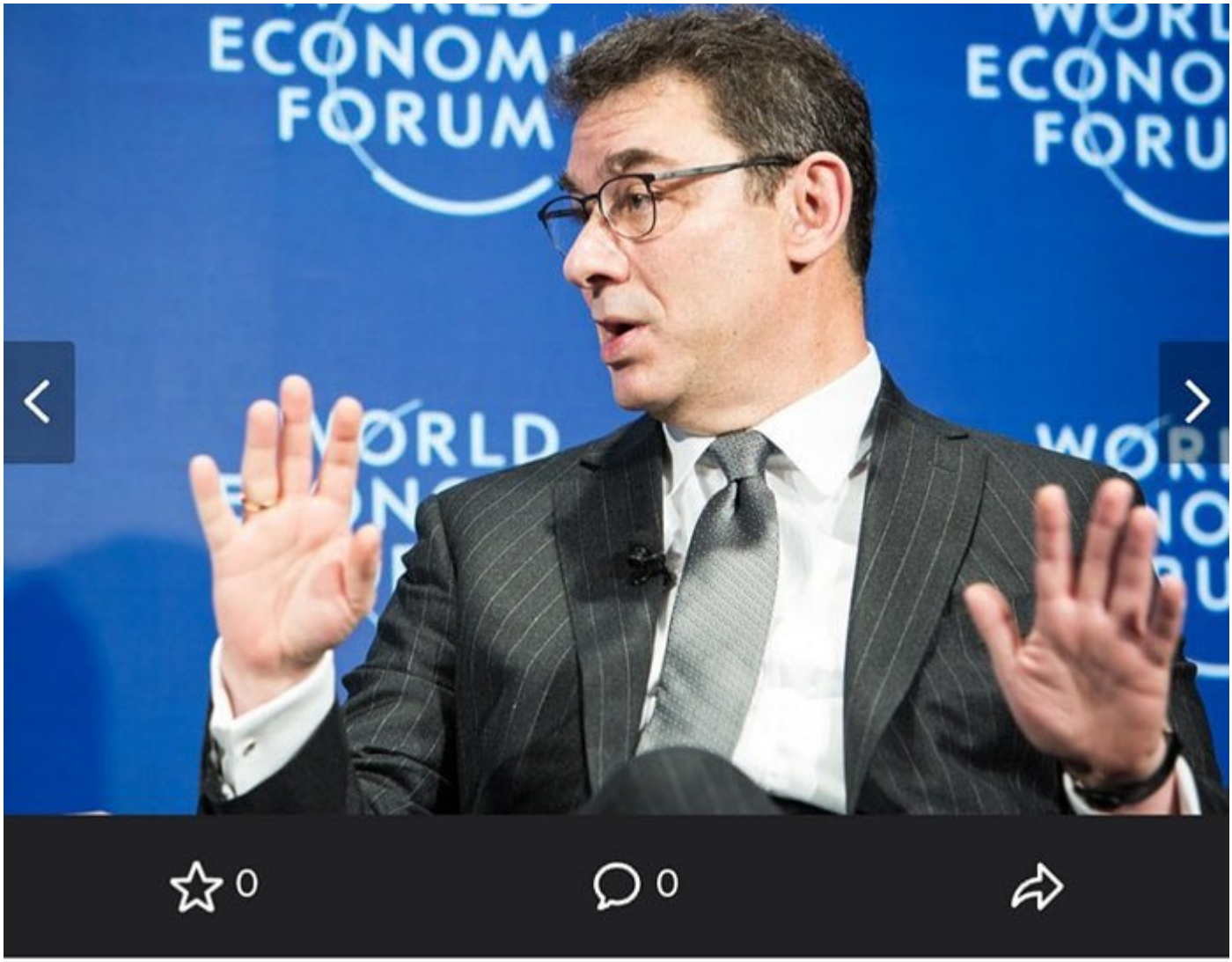


A molecular model of the spike proteins (red) of SARS-CoV-2 binding to the angiotensin-converting enzyme 2 (ACE2) protein, the receptor (blue) which is its the entry route to the target cell. Juan Gaertner/Science Photo Library

Albert Borula - WEF Agenda Contributor on “Future of Health and Healthcare”:
https://t.co/yIGrPiRm00

5 key trends for the future of healthcare (2018):
https://t.co/7dpo7CAgtr
- Gene Therapy
- Personalised medicine
- AI
- Wearables
https://t.co/70yqOzp7jR

H/t: @BFauker



World Economic Forum

PRO

Transforming Health in the Fourth Industrial Revolution

Albert Bourla , Chief Operating Officer, Pfizer, USA capture during the Session: Transforming Health in the Fourth Industrial Revolution at the Annual Meeting 2018 of the World Economic Forum in Davos, January 24, 2018
Copyright by World Economic Forum / Sikarin Thanachaiary

Potential role of ACE2-related microRNAs in COVID-19's nephropathy (2020): https://t.co/d0rdTOyM5D

"Based on this review, most of the ACE2-associated miRNAs affected multiorgans, therefore, their antimir therapy potentially lead to some adverse reactions."
https://t.co/tZxCQMZ4Yz

Noncoding RNA Res. 2020 Dec; 5(4): 153–166.
Published online 2020 Sep 9. doi: 10.1016/j.ncrna.2020.09.001
PMCID: PMC7480227
PMID: 32923747

Potential role of ACE2-related microRNAs in COVID-19-associated nephropathy

Ahmedz Widiasta,[a,b,*] Yunia Sribudiani,[b,c] Husna Nugrahapraja,[d] Dany Hilmanto,[a] Nanan Sekarwana,[a] and Dedi Rachmadi[a,b]

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Abstract

Go to:

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for coronavirus disease (COVID-19), potentially have severe kidney adverse effects. This organ expressed angiotensin-converting enzyme 2 (ACE2), the transmembrane protein which facilitate the entering of the virus into the cell. Therefore, early detection of the kidney manifestations of COVID-19 is crucial. Previous studies showed ACE2 role in various indications of this disease, especially in kidney effects. The MicroRNAs (miRNAs) in this organ affected ACE2 expression. Therefore, this review aims at summarizing the literature of a novel miRNA-based therapy and its potential applications in COVID-19-associated nephropathy. Furthermore, previous studies were analyzed for the kidney manifestations of COVID-19 and the miRNAs role that were published on the online databases, namely MEDLINE (PubMed) and Scopus. Several miRNAs, particularly miR-18 (which was upregulated in nephropathy), played a crucial role in ACE2 expression. Therefore, the antimiR-18 roles were summarized in various primate models that aided in developing the therapy for ACE2 related diseases.

Keywords: ACE2, microRNAs, COVID-19, Nephropathy

Neurons secrete miR-132-containing exosomes to regulate brain vascular integrity (April 2017):
https://t.co/SKLPDrdc3A
"Reduced expression of miR-132 by co-injection of
Cas9 mRNA and miR-132 gRNA."
|
"miR-132 have relative evidence as ACE2 gene silencer."
https://t.co/4QAMzykDZb

Open Access | Published: 21 April 2017

Neurons secrete *miR-132*-containing exosomes to regulate brain vascular integrity

Bing Xu, Yu Zhang, Xu-Fei Du, Jia Li, Hua-Xing Zi, Ji-Wen Bu, Yong Yan, Hua Han & Jiu-Lin Du

Cell Research **27**, 882–897 (2017) | Cite this article

14k Accesses | **134** Citations | **5** Altmetric | Metrics

Abstract

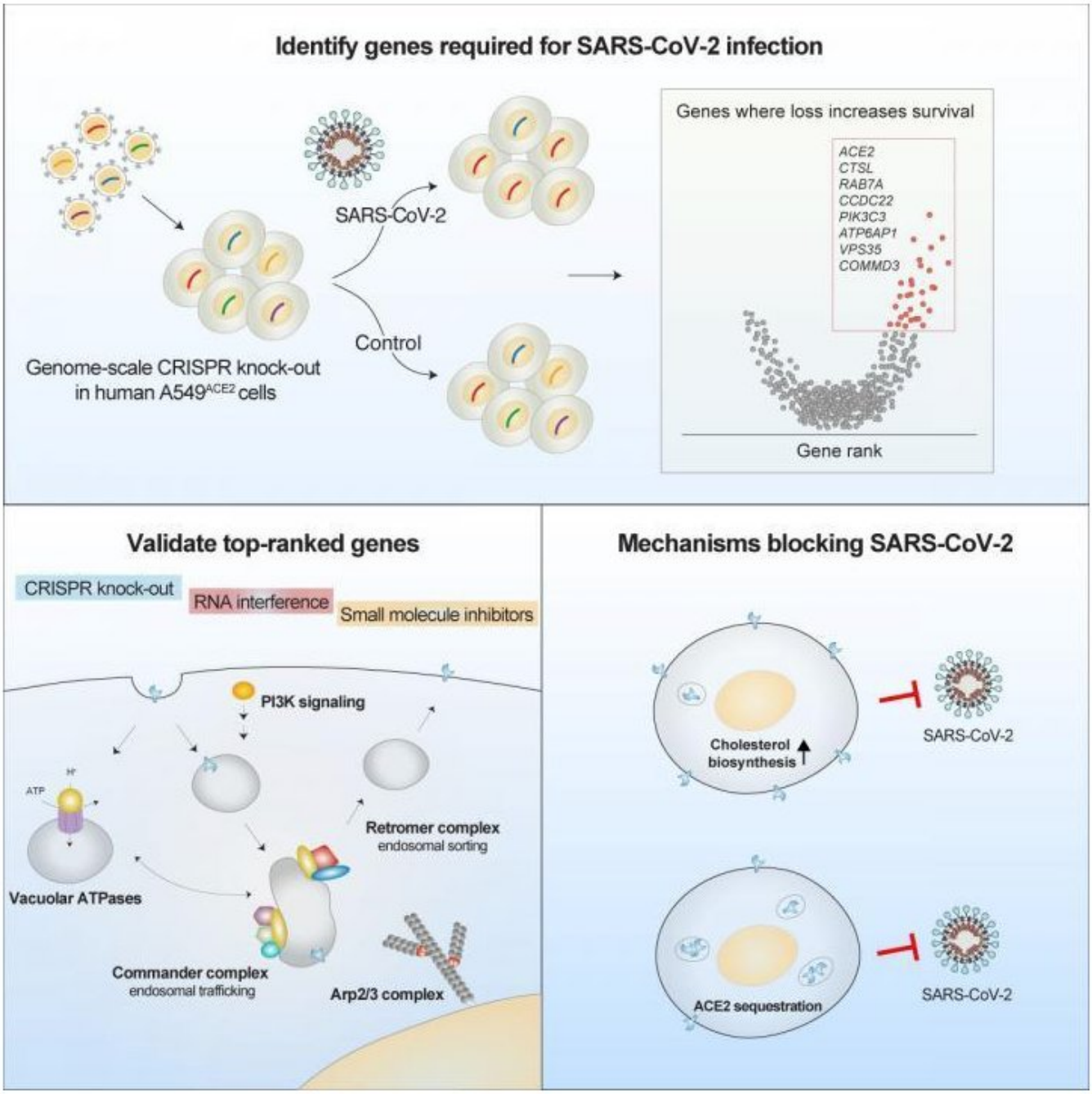
Vascular integrity helps maintain brain microenvironment homeostasis, which is critical for the normal development and function of the central nervous system. It is known that neural cells can regulate brain vascular integrity. However, due to the high complexity of neurovascular interactions involved, understanding of the neural regulation of brain vascular integrity is still rudimentary. Using intact zebrafish larvae and cultured rodent brain cells, we find that neurons transfer *miR-132*, a highly conserved and neuron-enriched microRNA, via secreting exosomes to endothelial cells (ECs) to maintain brain vascular integrity. Following translocation to ECs through exosome internalization, *miR-132* regulates the expression of vascular endothelial cadherin (VE-cadherin), an important adherens junction protein, by directly targeting *eukaryotic elongation factor 2 kinase* (*eef2k*). Disruption of neuronal *miR-132* expression or exosome secretion, or overexpression of vascular *eef2k* impairs VE-cadherin expression and brain vascular integrity. Our study indicates that *miR-132* acts as an intercellular signal mediating neural regulation of the brain vascular integrity and suggests that the neuronal exosome is a novel avenue for neurovascular communication.

NY Genome Center, NYU - CRISPR Screen Identifies Genes, Therapeutic Targets to Protect From Coronavirus Infection (October 2020):
https://t.co/KRxvc7Njok
"whether loss of some genes might confer resistance to the coronavirus by lowering ACE2 levels"
https://t.co/Cs6uwy8HfE

A team of researchers at the New York Genome Center, New York University, and the Icahn School of Medicine at Mount Sinai to identify new potential therapeutic targets for novel coronavirus has performed a genome-scale, loss-of-function CRISPR screen to systematically knock out all genes in the human genome.

The team examined which genetic modifications made human lung cells more resistant to viral infection. Their findings revealed individual genes and gene regulatory networks in the human genome that are required by SARS-CoV-2 and that confer resistance to viral infection when suppressed. The collaborative study described a wide array of genes that have not previously been considered as therapeutic targets for SARS-CoV-2. Their study was published online by *Cell* on October 24.

Link Between Host and Virus Genetic Dependencies



Graphical abstract of the Cell study (Sanjana Lab of New York Genome Center/New York University)

Transcriptional Inhibition of Host Viral Entry Proteins as a Therapeutic Strategy for SARS-CoV-2 (March 2020):
https://t.co/XkBFFdt4c1
https://t.co/Cs6uwy8HfE

Preprint **Article** *Version 1* **Preserved in Portico** **This version is not peer-reviewed**

Transcriptional Inhibition of Host Viral Entry Proteins as a Therapeutic Strategy for SARS-CoV-2

Xinchen Wang *, **Ryan Dhindsa** , **Gundula Povysil** , **Anthony Zoghbi** , **Joshua Motelow** , **Joseph Hostyk** , **David Goldstein**

Version 1 : Received: 24 March 2020 / Approved: 24 March 2020 / Online: 24 March 2020 (14:26:57 CET)
Version 2 : Received: 27 April 2020 / Approved: 28 April 2020 / Online: 28 April 2020 (09:39:02 CEST)

How to cite: Wang, X.; Dhindsa, R.; Povysil, G.; Zoghbi, A.; Motelow, J.; Hostyk, J.; Goldstein, D. Transcriptional Inhibition of Host Viral Entry Proteins as a Therapeutic Strategy for SARS-CoV-2. *Preprints* **2020**, 2020030360 (doi: 10.20944/preprints202003.0360.v1). Copy

Abstract

There is an urgent need to identify effective therapies for COVID-19 given that a broadly available and effective vaccine is likely at least one year away. Here, we identify compounds that transcriptionally inhibit host proteins required for SARS-CoV-2 entry and should be evaluated for efficacy in SARS-CoV-2 viral infection assays. Recognizing the need for immediately available treatment options, we focused particular attention on FDA-approved drugs that could be immediately repurposed to treat COVID-19 patients. By mining publicly available gene expression data, we identify several compounds that down-regulate *TMPRSS2*, a protein required for SARS-CoV-2 entry that has emerged as a promising therapeutic target. Among these, we find twenty independent studies that implicate estrogen-related and androgen-related compounds as transcriptional modulators of *TMPRSS2* expression, suggesting that these drugs and others acting on the pathway may be promising therapeutic candidates for COVID-19 for further testing. It is also noteworthy that *TMPRSS2* has highly variable and skewed expression in humans, spanning two orders of magnitude with a small minority of individuals having extremely high expression. Combined with literature showing that *TMPRSS2* loss-of-function in mouse is protective against SARS while anti-estrogen treatment predicted to increase *TMPRSS2* expression exacerbates SARS, this observation raises the hypothesis that *TMPRSS2* expression may positively correlate with severity in COVID-19.

Keywords

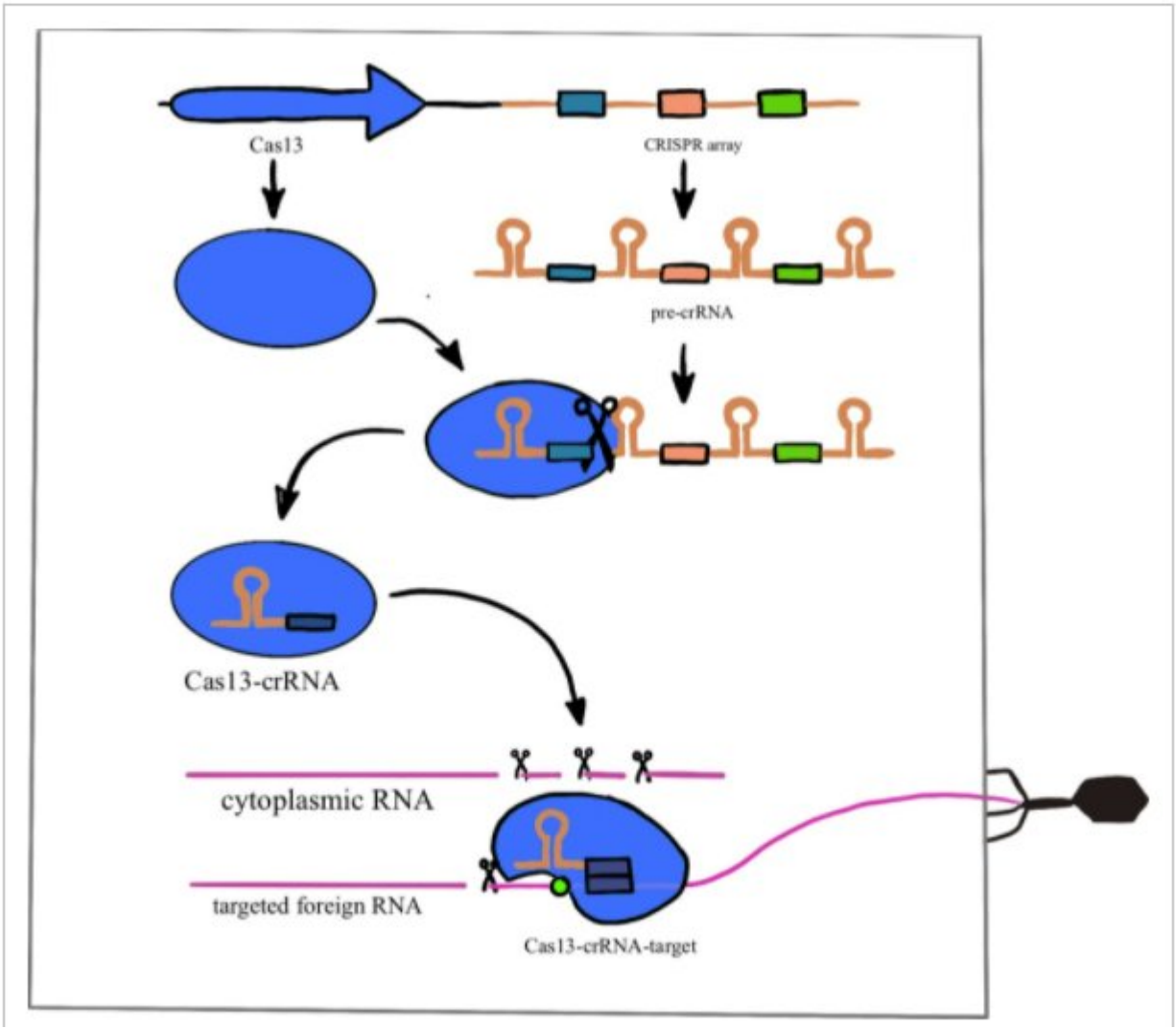
SARS-CoV-2; transcriptional inhibition; COVID-19; drug repurposing; TMPRSS2

A Guide to COVID-19 (May 23, 2020):
https://t.co/tREdKIsDQc
"authors were undergraduate students in a discovery-based research laboratory focused on projects centered on performing novel CRISPR-Cas9 genetic knockouts in zebrafish at Vanderbilt University"
https://t.co/LbfjcZsWck

Other possible treatments for COVID-19

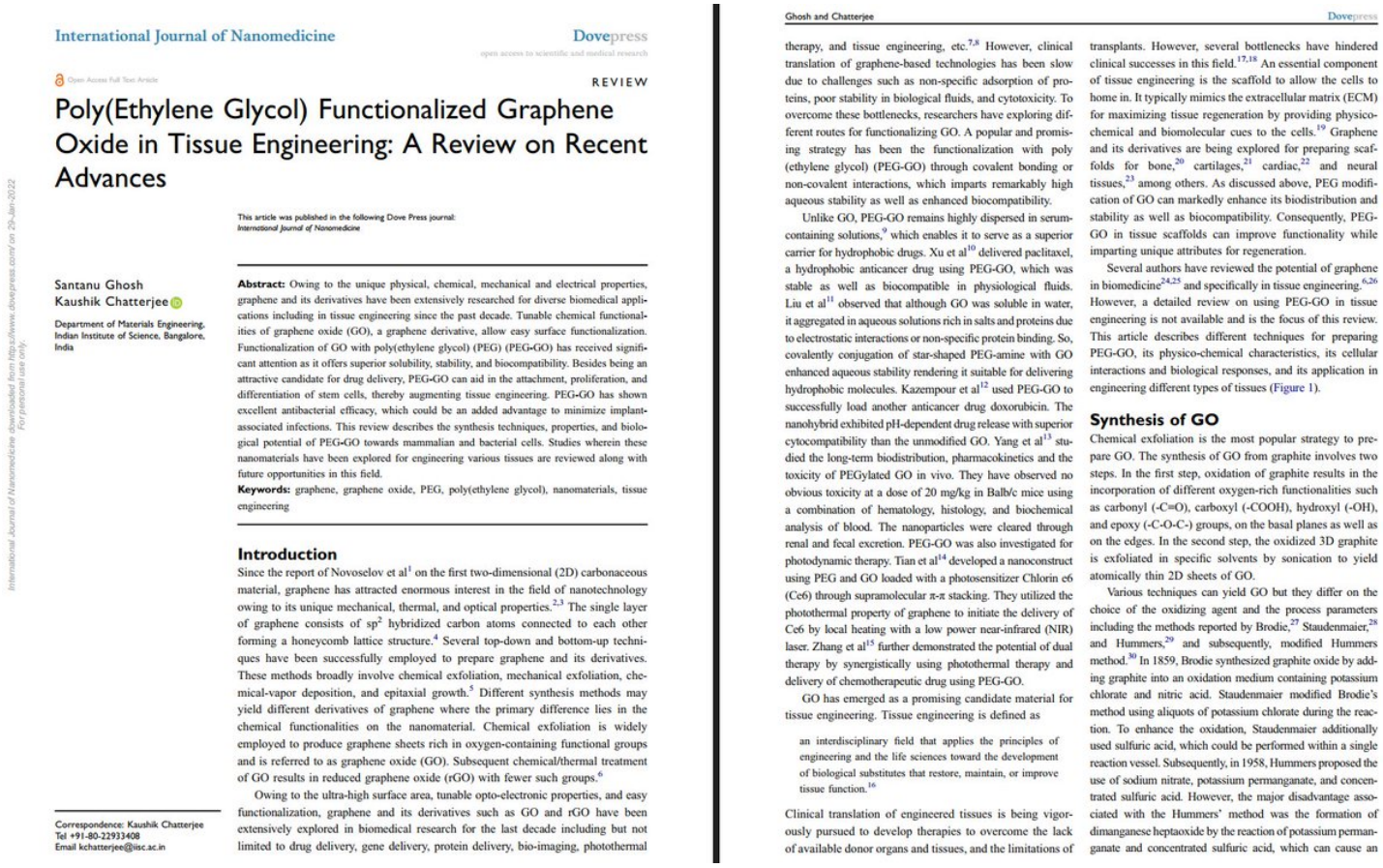
CRISPR

Along with possible drugs, new technologies have been utilized preemptively to counter the symptoms of SARS-CoV-2. CRISPR-Cas13 has been utilized to target essential parts of the SARS-CoV-2 virus, through an approach called PACMAN (Prophylactic Antiviral CRISPR in huMAN cells) [[138]]. Similar to SHERLOCK-based virus detection, PACMAN utilizes Cas13, which has RNase activity that can be used for both the detection (see above) and the destruction of SARS-CoV-2 (Fig. 4) [[139]]. This aspect of Cas13 can be utilized to destroy the virus with pinpoint accuracy, while also knocking out the RdRps. The main roadblock to using this technology is delivery. Some of the problems associated with the delivery of CRISPR-Cas13 have been researched, and possible liposomal delivery systems have been established [[138]]. While these treatments still require further testing, there is promising research in the use of CRISPR as a means of targeting the virus and stopping symptoms, as well as utilizing PACMAN to prevent future pandemics.



PEG-GO, Poly(Ethylene Glycol) Functionalized Graphene Oxide, in Tissue Engineering: A Review on Recent Advances (April 2020):
https://t.co/5Vh1UL0b4F

https://t.co/0BkwNvzHwc



International Journal of Nanomedicine

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REVIEW

Poly(Ethylene Glycol) Functionalized Graphene Oxide in Tissue Engineering: A Review on Recent Advances

This article was published in the following Dove Press journal:
International Journal of Nanomedicine

Santanu Ghosh
Kaushik Chatterjee

Department of Materials Engineering, Indian Institute of Science, Bangalore, India

Abstract: Owing to the unique physical, chemical, mechanical and electrical properties, graphene and its derivatives have been extensively researched for diverse biomedical applications including in tissue engineering since the past decade. Tunable chemical functionalities of graphene oxide (GO), a graphene derivative, allow easy surface functionalization. Functionalization of GO with poly(ethylene glycol) (PEG) (PEG-GO) has received significant attention as it offers superior solubility, stability, and biocompatibility. Besides being an attractive candidate for drug delivery, PEG-GO can aid in the attachment, proliferation, and differentiation of stem cells, thereby augmenting tissue engineering. PEG-GO has shown excellent antibacterial efficacy, which could be an added advantage to minimize implant-associated infections. This review describes the synthesis techniques, properties, and biological potential of PEG-GO towards mammalian and bacterial cells. Studies wherein these nanomaterials have been explored for engineering various tissues are reviewed along with future opportunities in this field.

Keywords: graphene, graphene oxide, PEG, poly(ethylene glycol), nanomaterials, tissue engineering

Introduction

Since the report of Novoselov et al[1] on the first two-dimensional (2D) carbonaceous material, graphene has attracted enormous interest in the field of nanotechnology owing to its unique mechanical, thermal, and optical properties.[2,3] The single layer of graphene consists of sp^2 hybridized carbon atoms connected to each other forming a honeycomb lattice structure.[4] Several top-down and bottom-up techniques have been successfully employed to prepare graphene and its derivatives. These methods broadly involve chemical exfoliation, mechanical exfoliation, chemical-vapor deposition, and epitaxial growth.[5] Different synthesis methods may yield different derivatives of graphene where the primary difference lies in the chemical functionalities on the nanomaterial. Chemical exfoliation is widely employed to produce graphene sheets rich in oxygen-containing functional groups and is referred to as graphene oxide (GO). Subsequent chemical/thermal treatment of GO results in reduced graphene oxide (rGO) with fewer such groups.[6]

Owing to the ultra-high surface area, tunable opto-electronic properties, and easy functionalization, graphene and its derivatives such as GO and rGO have been extensively explored in biomedical research for the last decade including but not limited to drug delivery, gene delivery, protein delivery, bio-imaging, photothermal therapy, and tissue engineering, etc.[7,8] However, clinical translation of graphene-based technologies has been slow due to challenges such as non-specific adsorption of proteins, poor stability in biological fluids, and cytotoxicity. To overcome these bottlenecks, researchers have exploring different routes for functionalizing GO. A popular and promising strategy has been the functionalization with poly (ethylene glycol) (PEG-GO) through covalent bonding or non-covalent interactions, which imparts remarkably high aqueous stability as well as enhanced biocompatibility.

Unlike GO, PEG-GO remains highly dispersed in serum-containing solutions,[9] which enables it to serve as a superior carrier for hydrophobic drugs. Xu et al[10] delivered paclitaxel, a hydrophobic anticancer drug using PEG-GO, which was stable as well as biocompatible in physiological fluids. Liu et al[11] observed that although GO was soluble in water, it aggregated in aqueous solutions rich in salts and proteins due to electrostatic interactions or non-specific protein binding. So, covalently conjugation of star-shaped PEG-amine with GO enhanced aqueous stability rendering it suitable for delivering hydrophobic molecules. Kazempour et al[12] used PEG-GO to successfully load another anticancer drug doxorubicin. The nanohybrid exhibited pH-dependent drug release with superior cytocompatibility than the unmodified GO. Yang et al[13] studied the long-term biodistribution, pharmacokinetics and the toxicity of PEGylated GO in vivo. They have observed no obvious toxicity at a dose of 20 mg/kg in Balb/c mice using a combination of hematology, histology, and biochemical analysis of blood. The nanoparticles were cleared through renal and fecal excretion. PEG-GO was also investigated for photodynamic therapy. Tian et al[14] developed a nanoconstruct using PEG and GO loaded with a photosensitizer Chlorin e6 (Ce6) through supramolecular π-π stacking. They utilized the photothermal property of graphene to initiate the delivery of Ce6 by local heating with a low power near-infrared (NIR) laser. Zhang et al[15] further demonstrated the potential of dual therapy by synergistically using photothermal therapy and delivery of chemotherapeutic drug using PEG-GO.

GO has emerged as a promising candidate material for tissue engineering. Tissue engineering is defined as

> an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function.[16]

Clinical translation of engineered tissues is being vigorously pursued to develop therapies to overcome the lack of available donor organs and tissues, and the limitations of transplants. However, several bottlenecks have hindered clinical successes in this field.[17,18] An essential component of tissue engineering is the scaffold to allow the cells to home in. It typically mimics the extracellular matrix (ECM) for maximizing tissue regeneration by providing physico-chemical and biomolecular cues to the cells.[19] Graphene and its derivatives are being explored for preparing scaffolds for bone,[20] cartilages,[21] cardiac,[22] and neural tissues,[23] among others. As discussed above, PEG modification of GO can markedly enhance its biodistribution and stability as well as biocompatibility. Consequently, PEG-GO in tissue scaffolds can improve functionality while imparting unique attributes for regeneration.

Several authors have reviewed the potential of graphene in biomedicine[24,25] and specifically in tissue engineering.[6,26] However, a detailed review on using PEG-GO in tissue engineering is not available and is the focus of this review. This article describes different techniques for preparing PEG-GO, its physico-chemical characteristics, its cellular interactions and biological responses, and its application in engineering different types of tissues (Figure 1).

Synthesis of GO

Chemical exfoliation is the most popular strategy to prepare GO. The synthesis of GO from graphite involves two steps. In the first step, oxidation of graphite results in the incorporation of different oxygen-rich functionalities such as carbonyl (-C=O), carboxyl (-COOH), hydroxyl (-OH), and epoxy (-C-O-C-) groups, on the basal planes as well as on the edges. In the second step, the oxidized 3D graphite is exfoliated in specific solvents by sonication to yield atomically thin 2D sheets of GO.

Various techniques can yield GO but they differ on the choice of the oxidizing agent and the process parameters including the methods reported by Brodie,[27] Staudenmaier,[28] and Hummers,[29] and subsequently, modified Hummers method.[30] In 1859, Brodie synthesized graphite oxide by adding graphite into an oxidation medium containing potassium chlorate and nitric acid. Staudenmaier modified Brodie's method using aliquots of potassium chlorate during the reaction. To enhance the oxidation, Staudenmaier additionally used sulfuric acid, which could be performed within a single reaction vessel. Subsequently, in 1958, Hummers proposed the use of sodium nitrate, potassium permanganate, and concentrated sulfuric acid. However, the major disadvantage associated with the Hummers' method was the formation of dimanganese heptaoxide by the reaction of potassium permanganate and concentrated sulfuric acid, which can cause an

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Email kchatterjee@iisc.ac.in

CRISPR 2.0: Base Editing (BE) in the Groove (March 2021):
"Researchers from the Broad Institute, the NIH, and Vanderbilt University have used an adenine base editor (ABE) to treat progeria in mice."
https://t.co/ZktPEIePjS

https://t.co/Mi0Q6e9cTw

CRISPR 2.0: Base Editing in the Groove

A series of exciting preclinical and animal model results show that base editing is making rapid strides toward the clinic

By **Anjali A. Sarkar, PhD** - March 4, 2021 0

Researchers from the Broad Institute, the NIH, and Vanderbilt University have used an adenine base editor (ABE) to treat progeria in mice. Correcting the mutation that causes progeria led to strong symptom reduction and longer lifespan. An ABE is shown here bound to a guide RNA and a targeted piece of DNA. [Aditya Raguram, Liu Laboratory, Broad Institute]

A reasonable idea, suggests Sekar Kathiresan, MD, co-founder and CEO of Verve Therapeutics, is the development of a one-time treatment—a single spelling change in the DNA of a liver gene in an adult person—that would "turn off the gene and lower blood cholesterol levels for the rest of the person's life." Although this idea would not have been taken very seriously 10 years ago, it is now being investigated. It is a potent illustration of the magnificent promise of genome editing in general, and of base editing in particular.

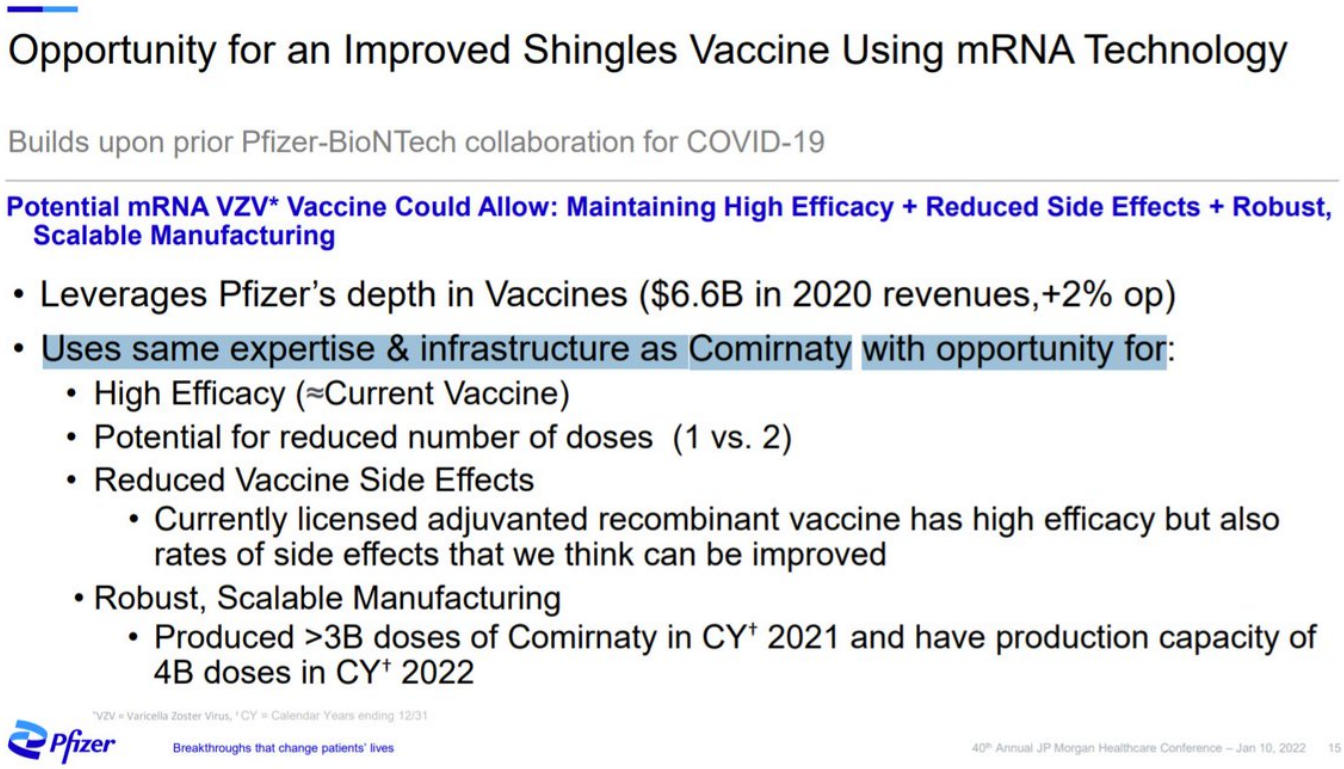
Millions of people manage heart disease with daily doses of cholesterol-lowering drugs such as statins. But a one-and-done approach is on the horizon thanks to base editing, a budding technology in the genome editing space. Since its first publication in *Nature* and *Science* in 2016, base editing has made rapid strides through preclinical studies and is poised to revolutionize medicine.

This precise technology holds the key for a new class of genetic medicines developed through direct correction of disease-causing mutations and other genetic manipulations—inserting protective genetic variants, activating or silencing regulatory elements, knocking out genes through stop codon or splice site mutations, and potentially realizing a multiplexed approach.

Recently Featured


Biogen Poised for Pipeline Growth, Samsung Eyes New Drug Expansion after $2.3B Joint Venture Deal

Pfizer CRISPR-Cas9 mRNA:
1. mRNA for Adenine Base Editing
2. guideRNA (gRNA)
3. Amino lipid
4. Cholesterol
5. DSPC
6. Poly(Ethylene Glycol)-lipid
https://t.co/9HwX63Ud7A



Pfizer, Albert Borula - Advancing Our mRNA Strategic Development (January 10, 2022):https://t.co/yJrJ2cZVvJ

Breakthrough that changes people\u2019s lives - CRISPR-Cas9, gene knockdowns

1. mRNA for Adenine Base Editing
2. guide RNA
3. Amino lipid
4. Cholesterol
5. DSPC
6. PEG Lipid https://t.co/2FrAx5ydxt pic.twitter.com/D0Oas7VTPH

— Very 0wn (@Very_0wn) January 27, 2022

"Moderna - Arbutus Biopharma LNP patent."
https://t.co/QNUKZ9oKNM

Arbutus Biopharma patents:
https://t.co/TAfaysB6kw
|
Delivering CRISPR therapeutics with lipid nanoparticles (2015):
https://t.co/xAgR5rwNqn
"to silence the expression of the target gene"

https://t.co/yFPl8bfxZw

Lipid Compositions App 20200138955 - Manoharan; Muthiah ; et al.	2020-05-07
Delivering CRISPR therapeutics with lipid nanoparticles Grant 10,626,393 - Lee , et al.	2020-04-21
Novel Lipid Formulations For Nucleic Acid Delivery App 20200113832 - YAWORSKI; Edward ; et al.	2020-04-16
Trialkyl cationic lipids and methods of use thereof Grant 10,561,732 - Heyes , et al. Feb	2020-02-18
Substituted 1-hydroxy-pyridin-2(1H)-ones, and methods of making and using same Grant 10,550,084 - Kakarla , et al. Fe	2020-02-04
Substituted Dihydroindene-4-carboxamides And Analogs Thereof, And Methods Using Same App 20200024226 - Cole; Andrew G. ; et al.	2020-01-23
Methods For Ameliorating Infusion Reactions App 20190328664 - BECHARD; Jeffrey P. ; et al.	2019-10-31
Lipid compositions Grant 10,456,473 - Manoharan , et al. Oc	2019-10-29
Substituted Pyridinone-containing Trycyclic Compounds, And Methods Using Same App 20190314347 - BAILEY; Danielle Lauren ; et al.	2019-10-17
Therapeutic Compositions And Methods For Treating Hepatitis B App 20190282604 - CUCONATI; Andrea ; et al.	2019-09-19
Compositions and methods for silencing hepatitis B virus gene expression Grant 10,415,037 - Cross , et al. Sept	2019-09-17
Compositions And Methods For Delivering Messenger Rna App 20190240354 - ABRAMS; Michael J. ; et al.	2019-08-08
Novel Lipid Formulations For Delivery Of Therapeutic Agents To Solid Tumors App 20190240339 - Yaworski; Ed ; et al.	2019-08-08