CRISPR-Cas9 Gene Editing Potential in Cancer Therapeutics

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Introduction/Abstract

The American Cancer Society (ACS) predicts that 1.9 million new cancer diagnoses and 600,000 cancer mortalities will occur in 2023. In addition, cancer therapies such as chemotherapy are unable specifically target cancer stem cells resulting in harmful side effects to the patient. While targeted cancer therapy does exist, it is not an available treatment for all cancer types.

ACS Cancer Incidence & Mortality

Table 1. Estimated Number* of New Cancer Cases and Deaths by Sex, US, 2023

	Estimated New Cases			Estimated Deaths		
	Both sexes	Male	Female	Both sexes	Male	Female
All sites	1,958,310	1,010,310	948,000	609,820	322,080	287,740
Oral cavity & pharynx	54,540	39,290	15,250	11,580	8,140	3,440
Tongue	18,040	13,180	4,860	2,940	1,950	990
Mouth	14,820	8,680	6,140	3,090	1,870	1,220
Pharynx	20,070	16,340	3,730	4,140	3,260	880
Other oral cavity	1,610	1,090	520	1,410	1,060	350
Digestive system	348,840	194,980	153,860	172,010	99,350	72,660
Esophagus	21,560	17,030	4,530	16,120	12,920	3,200
Stomach	26,500	15,930	10,570	11,130	6,690	4,440
Small intestine	12,070	6,580	5,490	2,070	1,170	900
Colon & rectum†	153,020	81,860	71,160	52,550	28,470	24,080
Colon	106,970	54,420	52,550			
Rectum	46,050	27,440	18,610			
Anus, anal canal, & anorectum	9,760	3,180	6,580	1,870	860	1,010
Liver & intrahepatic bile duct	41,210	27,980	13,230	29,380	19,000	10,380
Gallbladder & other biliary	12,220	5,750	6,470	4,510	1,900	2,610
Pancreas	64,050	33,130	30,920	50,550	26,620	23,930
Other digestive organs	8,450	3,540	4,910	3,830	1,720	2,110
Respiratory system	256,290	131,150	125,140	132,330	71,170	61,160
Larynx	12,380	9,900	2,480	3,820	3,070	750
Lung & bronchus	238,340	117,550	120,790	127,070	67,160	59,910
Other respiratory organs	5,570	3,700	1,870	1,440	940	500
Bones & joints	3,970	2,160	1,810	2,140	1,200	940
Soft tissue (including heart)	13,400	7,400	6,000	5,140	2,720	2,420
Skin (excluding basal & squamous)	104,930	62,810	42,120	12,470	8,480	3,990
Melanoma of the skin	97,610	58,120	39,490	7,990	5,420	2,570
Other nonepithelial skin	7,320	4,690	2,630	4,480	3,060	1,420
Breast	300,590	2,800	297,790	43,700	530	43,170

Fig. 1 Data table provided from the American Cancer Society's annual statistics journal. Cancer incidences and deaths are categorized by 'Male', 'Female', 'Both Sexes', and site of cancer formation

American Cancer Society. 2023. Cancer Facts & Figures. cancerorg. https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2023/2023-cancer-facts-and-figures.pdf.

Motive

- The motivation for this research was developed due to prior coursework knowledge of CRISPR-Cas9 and the basics of Cancer. This led to the question of how a genome editing tool could help treat a disease with genetic mutations at its core.

Method

- This literature review was conducted to explore the potential effectiveness and drawbacks of CRISPR in cancer therapies. This review also analyzed research in the efficacy of current cancer treatments to identify an area of treatment that could benefit from CRISPR

Acknowledgements

- I would like to thank Dr. Nahmani and Dr. Heller for their mentorship and support throughout the process. This was an insightful experience and helped shape decisions towards my future.

CRISPR-Cas9: General Function

CRISPR-Cas9 is composed of a Guide RNA (gRNA) in which the sequence can be customized to target a specific sequence. The Cas9 endonuclease then cuts the target sequence at the Protospacer Adjacent Motif (PAM) a portion of the gRNA sequence that aids in specificity.

CRISPR-Cas9: Limitations

A large concern of CRISPR is unintended alterations of DNA which could occur during the process or DNA repair. These consequences are known as off-target effects which could result in mutations or changes in sequence function. Another limitation of CRISPR would be the length of targetable sequence, which would require additionally CRISPR complexes to help increase specificity.

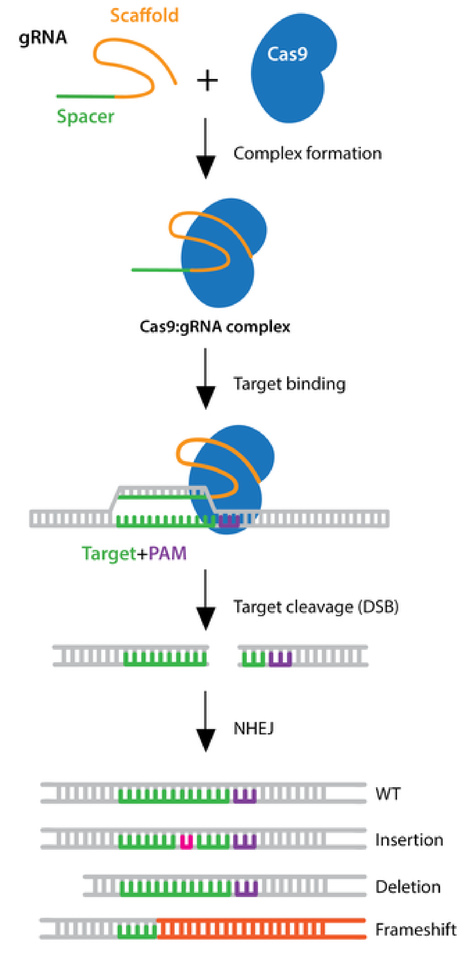

Fig. 2 Diagram showing the components of the CRISPR Complex. Demonstrates general function and potential outcomes after use.

Addgene. 2019. Addgene: CRISPR Guide. wwwaddgeneorg. https://www.addgene.org/guides/crispr/#?.

Results

- Cationic Lipids: Promising method of secured CRISPR complex transfer to human cells
- Customized gRNA could allow for personalized treatment, (i.e., differences in driver mutation sequence)

Cationic Lipid-Mediated Delivery

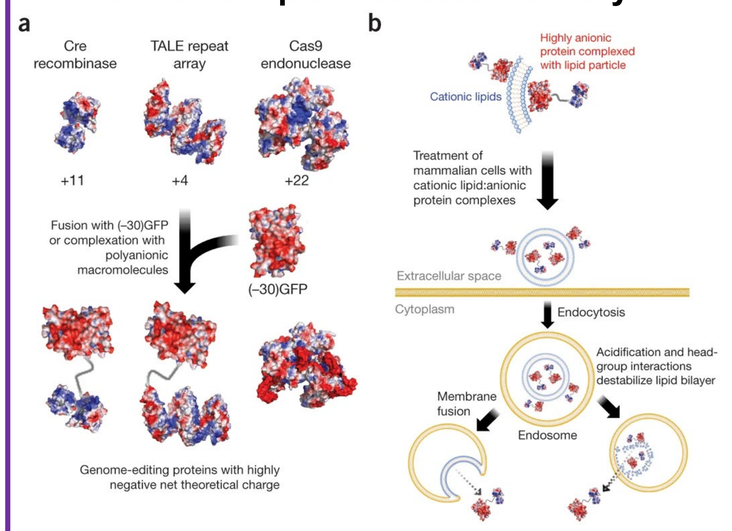

Fig. 4 Researcher's method of action for testing the transfer of proteins into mammalian cells.
(a) Displays 3 proteins of interest to be paired with an anionic GFP.
(b) Researcher's conceptual idea of cationic lipids would transfect the proteins into mammalian cells

Zuris JA, Thompson DB, Shu Y, Guilinger JP, Bessen JL, Hu JH, Maeder ML, Joung JK, Chen Z-Y, Liu DR. 2015. Efficient Delivery of Genome-Editing Proteins In Vitro and In Vivo. Nature biotechnology. 33(1):73–80. doi:https://doi.org/10.1038/nbt.3081. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4289409/.

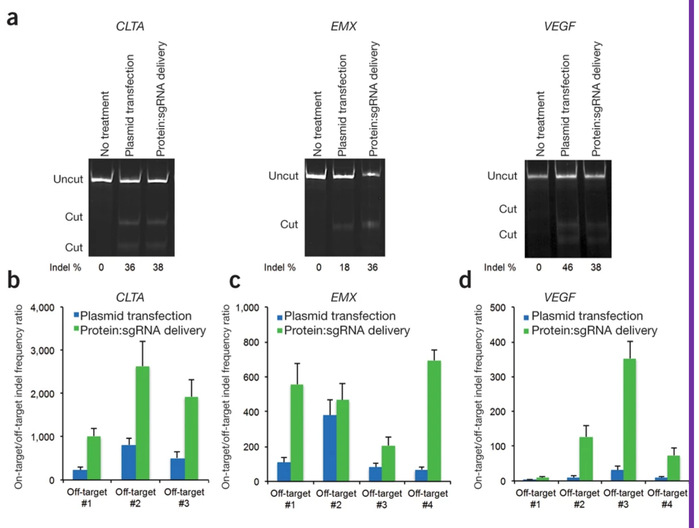

Fig. 5 Data from the researchers of figure 4. (a) Assay performed to compare gene modification performed by a RNAiMAX complex between plasmid transfection and Protein:sgRNA delivery. (b-d) Researcher's data showing the On-Target/Off-Target indel frequency ratio for selected gene sequences

Zuris JA, Thompson DB, Shu Y, Guilinger JP, Bessen JL, Hu JH, Maeder ML, Joung JK, Chen Z-Y, Liu DR. 2015. Efficient Delivery of Genome-Editing Proteins In Vitro and In Vivo. Nature biotechnology. 33(1):73–80. doi:https://doi.org/10.1038/nbt.3081. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4289409/.

Increase in CRISPR Sequence Specificity

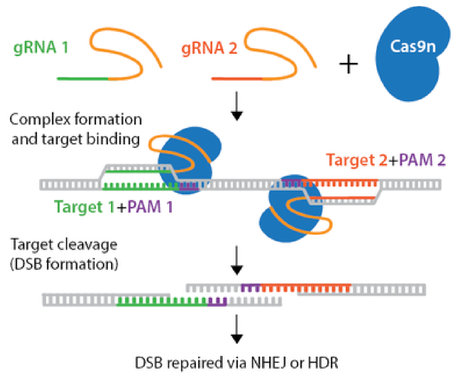

Fig. 3 Diagram shows another method CRISPR complexes which help mitigate limitations of target sequence length

Addgene. 2019. Addgene: CRISPR Guide. wwwaddgeneorg. https://www.addgene.org/guides/crispr/#?.

Conclusions

- The sequence targeting capability of CRISPR could allow for removal of oncogenes and repair tumor suppressor genes and could be used in tandem with other therapeutics increasing treatment efficacy. Further research is required to identify unforeseen side effects such as rate of 'off-target' effects from CRISPR as well as the efficacy of this method.

Future Directions

- Further Research into severity of off-target effects, and Efficacy of CRISPR knock-out/in
- Testing of Cationic Lipid permeability into tumors and Tumor Microenvironment response
- Progress towards a more in-depth genome data base could help increase sequence specificity and CRISPR efficacy.
- Applications of CRISPR in therapeutics for other diseases

References