Germline outline: **Using CRISPR to edit the germline in cancer patients**

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Problem

A genetic disease like Breast Cancer can be passed onto future generations and having a mutation of the BRCA 1 and BRCA 2 genres can increase the chances of developing breast cancer

Proposed Solution

Use micro injections of Cas 9 mRNA and sgRNA into zygotes in order to modify certain mutagenic alleles.

A zygote that had been fertilizer for 6 months would be injected with a certain amount of Cas 9 and sgRNA dependent on the amount of disrupt that is wanted to be achieved. Double nicking of the strand will occur by using a Cas 9 nickase. This would be done to increase efficiency by activating non homologous end joining (NHEJ)or Homology-Directed Repair(HDR). The sgRNAs would be a complement to opposite strands of the target site. HDR is performed specifically by inserting a donor oligonucleotide.

Zygote injection was successfully done on mice zygotes in 2013 and on human tripronuclear zygotes in 2015.

The injection in mice zygotes purposefully created mutations in a specified loci or allele.

This application could be done to edit the mutation of the BRCA1 genes which is sometimes responsible for malignant tumor growth.

Regulatory issues

NHEJ could possibly cause off target mutation since it does not have a homologous strand to copy. HDR in rare cases can also cause mutations due to the oligonucleotide.

There is no way to predict the effect these changes will have on future generations.

Proposed Regulation

Use double or paired nicking when cleaving with cas 9 so that the mutation can be efficiently changed in the zygotes dna.

References

Wang H., et. al. “One-step generation of mice carrying mutations in multiple genes by CRISPR/cas-mediated genome engineering.” Cell. 2013 May 9;153(4):910-8.

Injected sgRNA programmed for a specific loci also with Cas 9 mRNA to create mutations.

Ran F. A., et. Al. “Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity.” Cell, Volume 155, Issue 2, 10 October 2013, Pages 479-480

Used mutated Cas 9 nickase to increase efficiency of base repair

Liang, Puping et al. “CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes.” Protein & cell vol. 6,5 (2015): 363-372.

Experiment done on human zygotes

Wu Y. et. Al. “Correction of a genetic disease in mouse via use of CRISPR-Cas9.Cell Stem Cell.” 2013 Dec 5;13(6):659-62. doi: 10.1016/j.stem.2013.10.016.

Performed experiment on mice that corrected the germline, resulting mice were fertile and passed down the corrected alleles.

Torres D. et. al. “Prevalence and Penetrance of BRCA1 and BRCA2 Germline Mutations in Colombian Breast Cancer Patients.” Sci Rep. 2017 Jul 5;7(1):4713. doi: 10.1038/s41598-017-05056-y.

Review article discussing the penetrance or percentage of women that have the BRCA mutation.

Welcsh PL, et al. Inherited breast cancer: an emerging picture.Clin Genet. 1998 Dec;54(6):447-58.

Review article further explaining the BRCA genes and places suggestions on what is responsible for breast cancer.