Regulation of Cancer Stem Cells by Protein Post-Translational Modifications

Emily Khanh Pham, Dr. Hui Zhang
University of Nevada Las Vegas
College of Sciences

UNLV | CENTER FOR ACADEMIC ENRICHMENT & OUTREACH

AANAPISI

Abstract

Various diseases are caused by defective genes or mutations within the DNA. These mutations can cause cancer cells, which are usually treated through chemotherapy and radiation. However, these methods have not completely effective towards cancer stem cells, a group of cancer cells that possess stem cell properties and are capable of initiating cancer. In order to discover new methods of targeting these specific cells, technology for genome engineering will enable researchers to further study genetic requirements within these cells. The method of clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 will help cleave and alter genomic sequences to potentially correct the genomic mutations caused by the cancer stem cell. Using the technology of CRISPR-Cas9, studies of these cells and growth requirements can help discover new ways to target the cancer stem cell as a new method of therapy.

Introduction

The current research is on the roles of protein by post-translational modifications in cancer stem cells by generating a gene knockout mutation of genes that encode modifying activities involved in maintenance of cancer stem cells. This will lead to further studies of protein interactions in order to learn about the pathways within cancer cells. Based on the research, the development or discovery of a chemical inhibitor can be made.

The approach of this research can be completed by creating model cell lines to determine closely related gene functions and to find a way in correcting any genomic changes.[3] Numerous diseases, such as cancer in this case, are caused by a defective gene which can be a result even from a mutation in a single nucleotide.[6]

The development of genomic surgery is an approach that will be more specific to patients' method of treatment.[1] In order to target these mutations directly, studies are done by precisely cutting the target nucleotide sequence.[6] The type II clustered regularly interspaced short palindromic repeats (CRISPR) system uses a single endonuclease, Cas9. The enzyme cooperates with the guide RNA to specifically cleave invading DNA at sites separated by the proto-spacer adjacent motifs (PAMs).[2] The single guide RNA will program the Cas9 to create double strand breaks in the targeted DNA strand, making the specific cuts to further study the mutated proteins in the experiment.[2]

Methods

❖ **Aseptic Technique**

- This technique minimizes number of contaminants in the laboratory.[7]
- All surfaces and materials must be disinfected by spraying alcohol such as isopropanol or 70% ethanol while wearing proper equipment.[7]
- A Bunsen burner is ignited and must be adjusted so a blue cone appears on the flame to ensure the highest temperature is reached.[7] The heat created by the flame will rise up and cause microorganisms and dust particles to be forced outside of the sterile fume hood.[7]
- Heat all materials, which includes pipette tips, caps, bottles, and any other equipment that may cause contamination, before and after use.

❖ **Generate CRISPR Guide RNA into a plasmid for transfection into cells**

- 2 pairs of 20 oligonucleotides are designed and synthesized based 2 pairs NGG PAM sequence on DNA template sequence of targeted genes.[8] These pairs of oligonucleotides are made for two gRNAs. The process is performed twice, where oligo 3 and 4 are made for a different segment in the target gene with the PAM sequence.
- Target guide sequences are then cloned into the plasmid DNA carrier, the vector known as lentiGuide-Puro.[8]

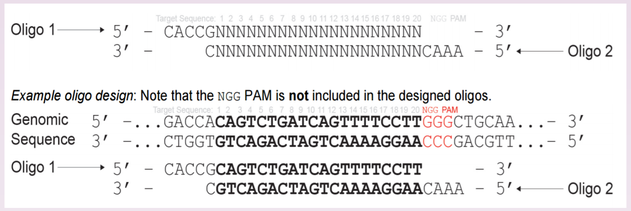

Figure 1: "Target Guide Sequence Cloning Protocol: In order to clone target sequence into the lentiGuide-Puro backbone, two oligonucleotides are synthesized. All plasmids will have same overhangs BsmBI digestion, therefore the oligonucleotides can be cloned into lentiCRISPRv2, lentiCRISPRv1, or lentiGuide-Puro." – ZhangLab[8]

❖ **Transfection Process**

- Using Puromycin drug for cells expressing Puro, transfect the guide-vectors in the cancer cells.[8]
- Cells will begin expressing the gRNA 1 from oligos 1 and 2 vector and gRNA 2 from oligos 3 and 4 vector.
- Cells will express lenticas9-blast, selected for blasticidin in the same cancer cell as the guide vectors selected for Puro.[8]
- Both gRNAs will anneal with the DNA templates at two locations of the target gene.
- The Cas9 endonuclease will cut on RNA DNA loci on the target DNA template to delete fragment in between the gRNAs.[8]

❖ **Obtain Cell Clones**

- Single cell clones with the deleted copies will be marked and be obtained by serial cell dilution.
- Verification of the deletion clones are made by observing appearance of gene deletion such as PCR or protein detection.[8]

Data Analysis

❖ ***Western Blot***

- Separate according to size, transfer to a solid support, and then mark the target protein using a proper primary and secondary antibody to view the results.[5] The results will help indicate which cell is expressing the protein in order to focus more on the interaction.

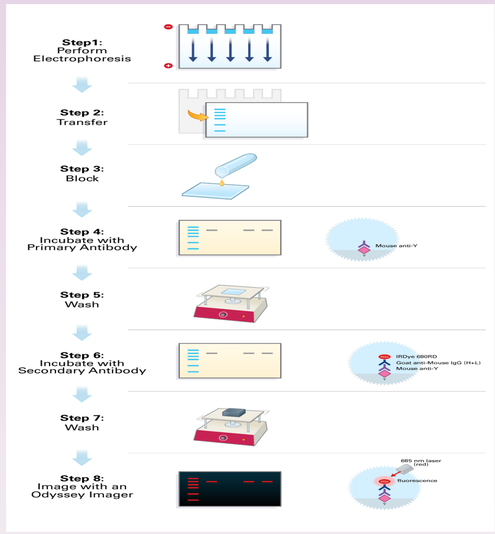

Figure 2: One-Color Workflow for Quantative Infrared Westerns: General steps in completing a Western Blot procedure.[5]

❖ ***DNA Electrophoresis***

- DNA is separated by their length in base pairs.
- The electrophoresis uses an electrical current to move the negatively charged DNA toward the positively charge area within the agarose gel created.

Conclusion

Developing an understanding and having the ability to assist in the process of forming a chemical inhibitor to treat diseases; in this case, cancer stem cell research can have a huge beneficial impact on this world. If there can be a new target of therapy towards cancer stem cells specifically, it can decrease the amount of damage to normal cells when patients undergo therapy.

Chemotherapy treatment does not guarantee a complete recovery and the small percentage of cancer stem cells that may exist could multiply within the next few years. Discovering a chemical inhibitor for treatment of therapy could be a safer and more effective alternative.

Due to the status of this research, no specific therapy or chemical inhibitor have been found. In order to reach this long term goal, the knock out cells' protein interaction and pathways must be closely studied in order to identify a target of therapy.

References

1. Brunicardi, F. C., Gibbs, R. A., Wheeler, D. A., Nemunaitis, J., Fisher, W., Goss, J., & Chen, C. (2011). Overview of the Development of Personalized Genomic Medicine and Surgery. *World Journal of Surgery*, *35*(8), 1693–1699. http://doi.org/10.1007/s00268-011-1056-0
2. Charpentier, E., & Doudna, J. A. (2013). Biotechnology: Rewriting a genome. *Nature, 495*(7439), 50-51. doi:10.1038/495050a
3. Cong, L., & Zhang, F. (2014). Genome Engineering Using CRISPR-Cas9 System. *Chromosomal Mutagenesis Methods in Molecular Biology,* 197-217. doi:10.1007/978-1-4939-1862-1_10
4. *LI-COR (2016).*Typical workflows for quantitative infrared western. [Chart]. Retrieved from https://www.licor.com/bio/applications/quantative_western_blots/workflows.html
5. Mahmood, T., & Yang, P.-C. (2012). Western Blot: Technique, Theory, and Trouble Shooting. *North American Journal of Medical Sciences*, *4*(9), 429–434. http://doi.org/10.4103/1947-2714.100998
6. Oost, J. V. (2013). New Tool for Genome Surgery. *Science, 339*(6121), 768-770. doi:10.1126/science.1234726
7. Sanders, E. R. (2012). Aseptic Laboratory Techniques: Volume Transfers with Serological Pipettes and Micropipettors. *Journal of Visualized Experiments : JoVE*, (63), 2754. Advance online publication. http://doi.org/10.3791/2754
8. Sanjana N., Shalem, O., Hartenian E., Shi, X., Scott, DA., Mikkelsen, T., Heckl, D., Ebert, BL., Root, DE., Doench, JG., Zhang, F. (2014). LentiCRISPRv2 and lentiGuide-Puro: lentiviral CRISPR/Cas9 and single guide RNA. *Genome-scale CRISPR Knock-Out* doi:10.1126/science.1247005