Applying CRISPR-Cas9 to Develop Sarcoma Models and Therapies

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For decades, oncology researchers have experienced difficulties finding reliable *in vitro* and *in vivo* cell models to study oncogenic fusions[1]. These translocations arise from repairing double-strand breaks (DSB) in non-homologous chromosomes, potentially leading to cancer development. Studies have shown that out of two repair pathways, Non-Homologous End Joining (NHEJ) and Homology Directed Repair (HDR), NHEJ is the leading pathway for generating such chromosomal rearrangements. Before the emergence of gene-editing tools such as CRISPR-Cas9, scientists modeled translocations in human cells and mouse models using fusion gene expressions - approaches that did not display entire chromosomal rearrangements and the underlying mechanism by which these translocations were produced. After the development of CRISPR-Cas9, scientists have been able to directly manipulate the NHEJ pathway using programmable nucleases[2], effectively modeling these mechanisms and producing cancer-related translocations in embryonic stem cells. These programmable nucleases can introduce a DSB at any locus[3] in the genome, and a template with a selectable marker is created and added at that site using the HDR pathway. A splice acceptor sequence is then used to express this promoterless selectable marker. Afterward, scientists add LoxP sequences on both ends of the selectable marker, and the fusion is introduced by the expression of Cre recombinase[4]. This approach was used to model EWSR1::WT1[5] in human embryonic stem-derived mesenchymal progenitors[6] (Figure 1). Generating these chromosomal translocations in stem cells using CRISPR has implications beyond studying translocation-associated tumors, possibly benefiting the field of regenerative medicine by making it possible for scientists to explore the histogenesis[7] of certain sarcomas and identify their cell of origin. Gene-editing tools have been proven beneficial in producing accurate and comprehensive *in vitro* and *in vivo* tumor models, making it possible for scientists to study cancer-related mutations and identify effective therapeutic strategies to combat them.

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[1] Mutations that lead to cancer development (may be a result of chromosomal deletion, insertion, or translocations)
[2] Enzyme that is cleaves a phosphodiester bond between the pentose of one nucleotide and the phosphate group of another in nucleic acids
[3] The specific location of a gene on a chromosome (singular - locus, plural - loci)
[4] An enzyme that will identify and delete the DNA sequence between LoxP sites in a genome.
[5] The chromosomal translocation associated with a sarcoma known as desmoplastic small round cell tumor
[6] Cell that is presumed to be the precursor of sarcoma tumors
[7] Development of specialized cells and organs from undifferentiated cells

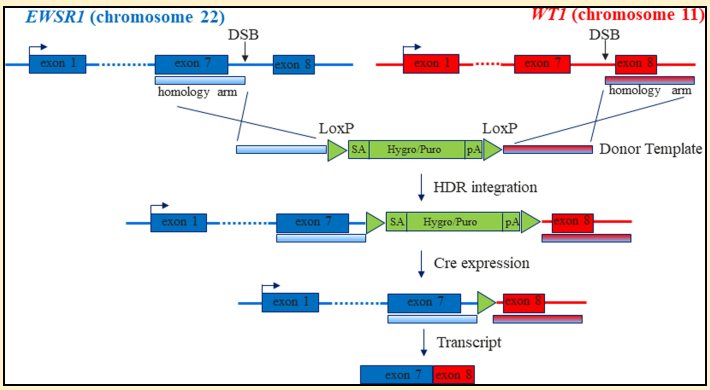
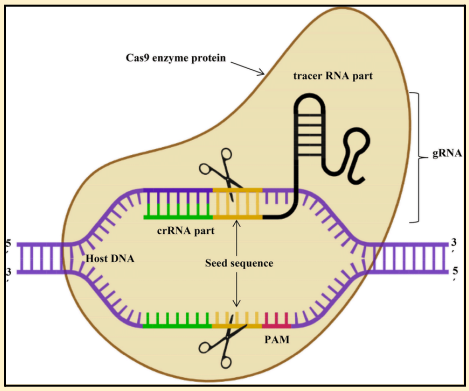


Figure 1 - Process in which EWSR1::WT1 chromosomal translocation and oncogenic fusion is induced, via gene editing. Source: National Institutes of Health (NIH)

In addition to using CRISPR-Cas9 to develop reliable sarcoma models, scientists have applied the technology to identify certain proteins and alter genes that play a role in sarcoma growth and development. One study analyzing osteosarcoma[8] cancer stem cells[9] (CSCs) used the CRISPR/Cas9 system to identify that a transcriptional factor[10] called Kruppel-like factor 11 (KLF11) regulates the survival and progression of osteosarcoma CSCs. KLF11's mechanism of action is to prevent the transcription of Yes Associated Protein (YAP), which promotes the transcription of oncogenes[11]. Moreover, KLF11 was found to be druggable as pharmacological activation enhanced a chemotherapy response, representing a possible avenue for therapeutic advancement. Another study examined CRISPR-Cas9 technology in eliminating Ewing sarcoma, a highly aggressive bone cancer characterized by a chromosomal translocation that results in the formation of EWSR1-FL1, a protein crucial for the growth of Ewing sarcoma's cancerous tumors. By targeting exon 9 of the Friend Leukemia Integration 1(FLI1) gene present in the Ewing sarcoma cells tested, the genome editing technology efficiently inactivated EWSR1-FL1, permanently setting the cells into the G1 phase. This resulted in severe cell growth arrest, and challenges in cell proliferation. These findings suggest that the CRISPR-Cas9 system upholds the potential to remain a humane and safe treatment to those affected by Ewing sarcoma.



These studies highlight the importance of using and understanding CRISPR-Cas9 screens and gene editing. In the KLF11 study, an entire human-genome scale CRISPR

[8] Most common type of bone cancer
[9] Cells that are able to reproduce indefinitely and differentiate into other cancer subtypes
[10] A protein that has a regulatory role in the transcription of other genes
[11] Genetic mutations that may cause cancer

knockout library, a sgRNA library, was used to screen, identify, and analyze KLF11 as a key suppressor of CSCs. These types of large-scale studies accelerate the discovery process of such proteins and regulators, and thus the development of drug targets. In the study relating to Ewing sarcoma, CRISPR-Cas9 technology actively edited the genome of Ewing sarcoma cells to completely destroy its cancerous cells. The results of these studies indicate that the CRISPR-Cas9 system is a more accommodating alternative to the widely used, but burdensome chemotherapy in fighting sarcoma patients. In addition to this, CRISPR-Cas9 has enabled scientists to accurately and reliably model translocation-associated tumors, bolstering the creation of treatments for oncogenic fusions.

References:

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Sources:

Image 2 - ResearchGate