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Oral Presentation

Application of CRISPR/Cas9 technique to the NRAS gene Q61K mutation in SK-MEL-30 skin cancer cell line

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Abstract

Malignant melanoma is a neoplasm of melanocytes or of the cells that develop from melanocytes. Although melanoma was once considered an uncommon disease, the annual incidence has increased dramatically over the past few decades, as have deaths from melanoma. The 3 Ras genes in humans (HRas, KRas, and NRas) are the most common oncogenes in human cancer; mutations that permanently activate Ras are found in 20% to 25% of all human tumors and up to 90% in certain types of cancer NRAS mutations in codons 12, 13, and 61 arise in 15–20 % of all melanomas. These alterations have been associated with aggressive clinical behavior and a poor prognosis. Until recently, there has been a paucity of promising genetically targeted therapy approaches for NRAS-mutant melanoma (and RAS-mutant malignancies in general). In this study, it was aimed to correct the Q61K mutation causing malignant melanoma cancer using the CRISPR / Cas9 technique, which is considered as one of the most effective techniques for genome editing. For this purpose, malignant melanoma SK-MEL-30 cell line containing the Q61K mutation was used. Once the gRNAs for the target mutation have been designed, they are transferred to plasmids and cloned. Then, plasmids and donor sequence were transferred to malignant melanoma skin cancer cells using electroporation technique. Successful transformed cells which are GFP + cells, sorted from other cells using fluorescence microscopy and flow cytometry. With the HDR-guided repair mechanism, knock-out and knock-in were targeted respectively. Real-time PCR analysis and deep-sequencing showed successful knock-out and knock-in in target cells in some cancer cells. In addition, end-point analysis supports the results of working successfully. In this project, it has been proved that even a point mutation can be corrected by the CRISPR / Cas9 technique. Using the CRISPR technique, we believe that we have given literature a new vision in terms of studying similar mutations.

Keywords: CRISPR/Cas9, Genome-editing, Malignant Melanoma, Q61K