CRISPR/Cas9 mediated knockout of GLI1, GLI2 and GLI3 genes in melanoma cell lines

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GLI transcription factors are the main mediators of Hedgehog-GLI (HH-GLI) signaling pathway. They activate the transcription of many target genes which are involved in various aspects of tumorigenesis. Targeting the HH-GLI signaling pathway is one of the recent approaches in cancer therapy. Our preliminary data suggest that melanoma cells harboring the BRAF mutation show a better response to GLI inhibition than cells with the NRAS mutation, suggesting a differential role for the HH signaling pathway in melanoma cells with different genetic background. In order to elucidate the role of GLI proteins in melanoma with these genetic backgrounds (BRAF mutation, NRAS mutation, no mutation), we are now in phase of constructing GLI1/2/3 knockout melanoma cell lines using CRISPR/Cas9 system. For this purpose, for each GLI protein, we designed two sgRNAs which guide the Cas9 protein to the specific sequences in the genome where they create a double stranded break. The designated sequence was the region near the ATG of GLI1/2/3 and the region near the end of the genes. These two breaks were repaired via homology-directed repair (HDR) with a help of HDR cassette that was transfected along with CRISPR/Cas9. So far we have managed to construct GLI2 knock-outs in two melanoma cell lines. In parallel, we have also over-exressed GLI1/2/3 proteins in the same melanoma cell lines. Each maternal cell line and its over-expressed cell line for GLI1, GLI2 and GLI3 will be analyzed by RNA-seq to determine the changes in transcriptomes. This analysis, in combination with knock-out cell line analysis results, should provide us with the information of which genes are specifically regulated by each of the GLI proteins in each of the genetic background. That may provide insight into the observed differences between the cell lines with different genetic background.