ABSTRACT

MicroRNAs (miRNAs) are a class of naturally occurring small non-coding RNAs that control gene expression to regulate a variety of developmental and physiological processes. The levels of miRNA expression vary between different human cell lines and tissues. For example, miRNA-373 and miRNA-520 are overexpressed in MCF7 breast cancer cells compared to A549 lung cancer cells, which may contribute to increased invasion and metastasis in breast cancer. In this study, targeted integration was used to deliver the miRNA genes into A549 and MCF7 cells to investigate the effect of miRNA expression on cell invasion and metastasis. The results showed that the expression of miRNA-373 and miRNA-520c in A549 and MCF7 cells using CompoZr™ Targeted Integration Technology. The CompoZr™ Targeted Integration Kit also allows for efficient targeted integration using a well-validated pair of six-finger nucleases (ZFN) designed to target the AAVS1 locus on human chromosome 19. An advantage of targeted integration is the elimination of the effects of genomic context on the expression of delivered transgenes. This technology allows for the expression of a user specified gene of interest and in this case miRNA genes.

INTRODUCTION

Zinc finger nucleases (ZFNs) are fusion proteins which contain zinc finger proteins and the non-nuclease specific domain of restriction enzyme FokI. Each zinc finger interacts with three nucleotides, and multiple (N) fingers can be associated together to specifically bind a targeted sequence of N base pairs. Fold must dimers to achieve double strand cleavage in the DNA. This means that a pair of ZFNs is required to bind and cut the targeted site (Figure 1). Specificity is determined by the number of fingers in the ZFNs. The ZFN is used to create a targeted double-strand break that stimulates the cell’s natural DNA repair processes of homologous recombination and Non-Homologous End Joining (NHEJ). These processes are harnessed to generate precisely targeted genomic edits, resulting in cell lines with targeted gene deletions, modifications, or integrations.

We presented data for the expression of human microRNA genes miR-373 and miR-520c in A549 and MCF7 cells using CompoZr™ Targeted Integration Technology. Here we present data for the expression of human microRNA genes miR-373 and miR-520c in A549 and MCF7 cells using CompoZr™ Targeted Integration Technology. In brief, total RNA was extracted from A549 and MCF7 cell lines via QuickExtract DNA Extraction Solution. These extracts were used in a junction PCR reaction (Jumpstart AccuTaq® LA DNA Polymerase Mix). Then these PCR products were cloned into pBluescript plasmid DNA and sequenced.

RESULTS

GAAGUGCUUC
GAUUUUGGGGUGU

ABCD

hsa-miR-520c expression to regulate a variety of developmental and physiological processes. The levels of miRNA expression vary between different human cell lines and tissues. For example, miRNA-373 and miRNA-520 are overexpressed in MCF7 breast cancer cells compared to A549 lung cancer cells, which may contribute to increased invasion and metastasis in breast cancer. In this study, targeted integration was used to deliver the miRNA genes into A549 and MCF7 cells to investigate the effect of miRNA expression on cell invasion and metastasis. The results showed that the expression of miRNA-373 and miRNA-520c in A549 and MCF7 cells using CompoZr™ Targeted Integration Technology. The CompoZr™ Targeted Integration Kit also allows for efficient targeted integration using a well-validated pair of six-finger nucleases (ZFN) designed to target the AAVS1 locus on human chromosome 19. An advantage of targeted integration is the elimination of the effects of genomic context on the expression of delivered transgenes. This technology allows for the expression of a user specified gene of interest and in this case miRNA genes.

CONCLUSIONS

• Successful integration of microRNA genes into the AAVS1 site in A549 and MCF7 cells
• Expression of functional human microRNAs 373 and 520c

FUTURE DIRECTIONS

• Express other Human miRNA genes and miRNA gene clusters
• Express miRNA Inhibitors
• Cell Migration
• SILAC (Stable isotope labelling with amino acids in cell culture)

REFERENCES

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