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CRISPR-Cas9 genome editing to evaluation the role of MMP9 in prostate cancer

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Introduction

Prostate cancer (PC), due to high prevalence, represents an important health problem, with strong economic impact. With the advancement of genome editing techniques using CRISPR-Cas9, possibilities for further studies in several neoplasms were found to better understand and control the mechanisms of cell resistance. Therefore, the oncogene MMP-9 play an important role in the process of migration and invasion of tumor cells to other tissues, generating metastases. However, these data are controversial. Due to the importance of these molecules in carcinogenesis, further investigation in PC is needed to evaluate the role of MMP-9 in PC, using CRISPR-Cas9 system.

Methods

MMP9 guide RNA (sgRNA) sequences were initially inserted into plasmid PX-330. These plasmids, with MMP-9 inserts, were transfected in PC cell lines DU145 and PC-3M-luc-C6. The gene and protein expression of MMP9 were performed by qRT-PCR and Western Blotting, respectively, and the apoptosis were analyzed by flow cytometry of the sgRNA transfected cells for MMP9 and compared to control group

Results

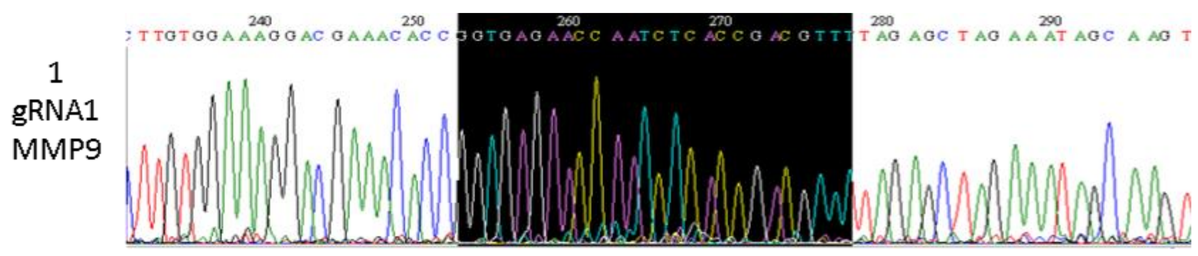

Figure 1. We performed the digestion and insertion techniques of MMP9 sgRNA 1 and sgRNA 2 of MMP9 in the PX-330 plasmid, validated by sequencing .

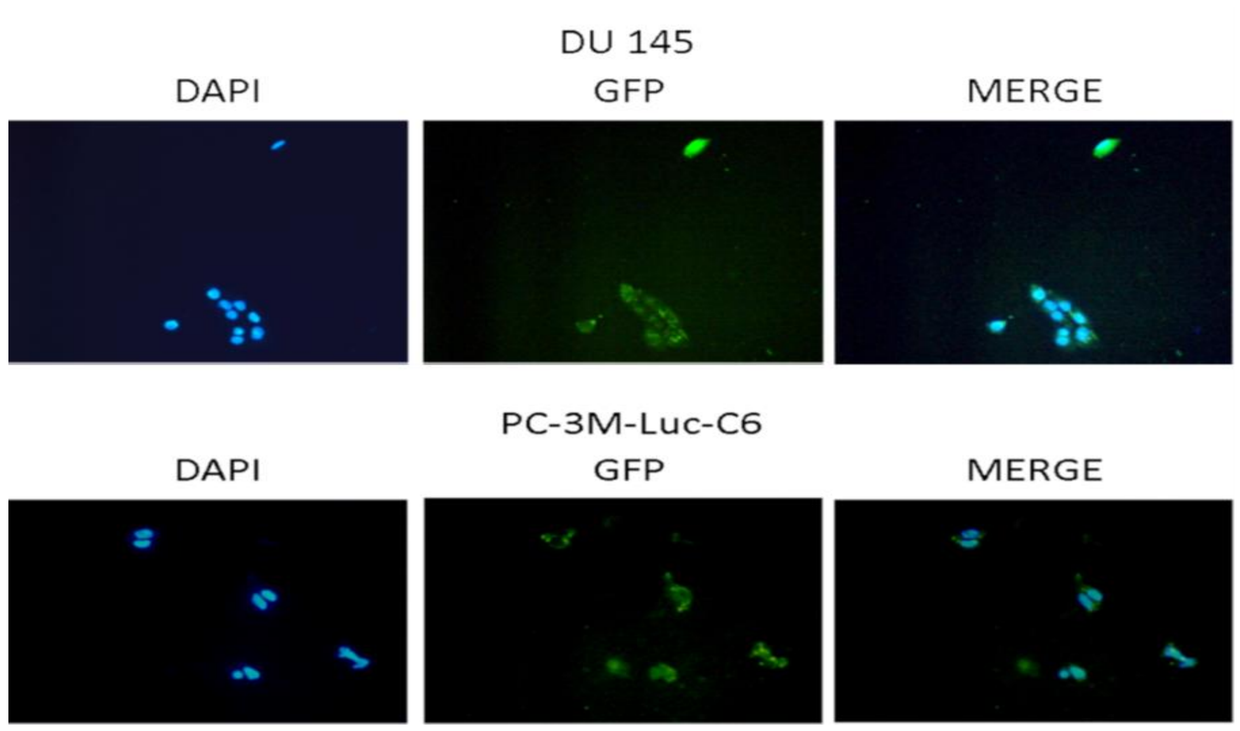

Figure 2. The transfected cells, DU 145 and PC-3M-Luc-C6 with MMP9 sgRNAs 1 and 2 showed positive GFP, that proves the transfection efficiency.

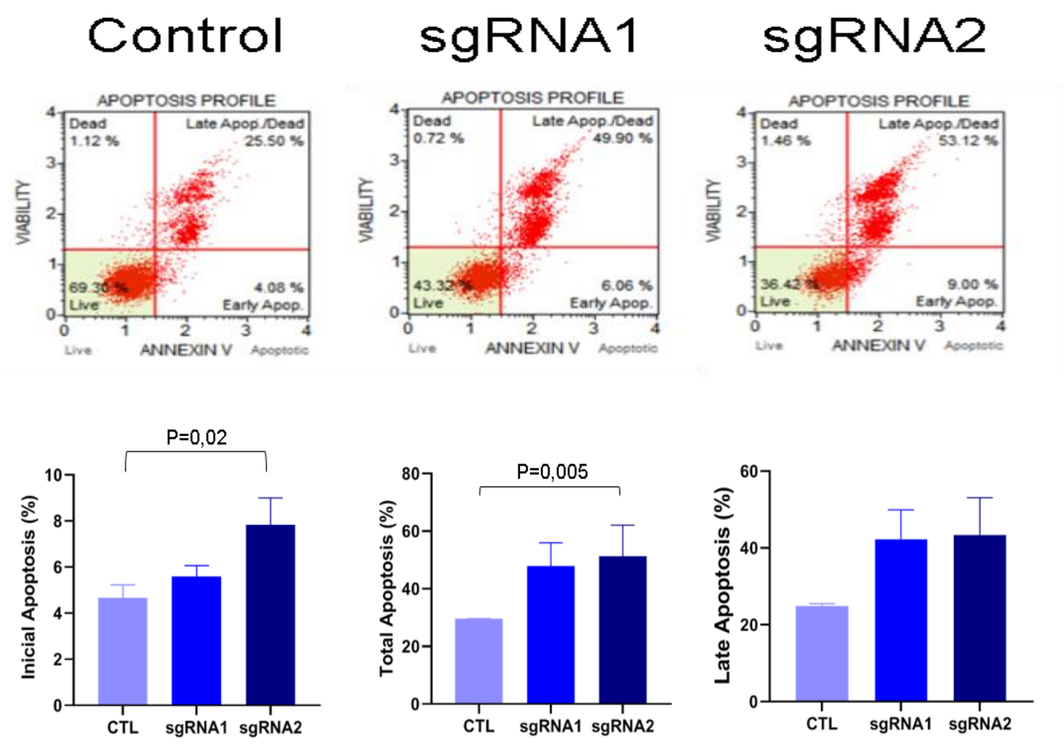

Figure 3. Increased initial apoptosis were observed in PC-3M-luc-C6 transfected with MMP9 sgRNA 1 and 2, p= 0,02, and total apoptosis, p=0,005, in compared to control cells.

Conclusion

The CRISPR-Cas9 system were effective, confirmed by the sequence results, and transfections by GFP. These cells transfected with sgRNAs 1 and 2 presented a higher apoptosis rate.

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