Effects of Lamin A/C Downregulation on Chromosome Territories 9 and 22 in Multiple Myeloma

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1. Introduction

Multiple myeloma (MM) is a currently incurable disease of the plasma cells[1]. MM is the second most frequent hematological malignancy[2]. Genomic instability and a heterogeneous phenotype of individual are observed in MM, which is due in part to the irregular pattern of gene expression, chromosome alterations and relative changes in chromosome territories (CT) compared to normal plasma cells[1,3]. The nuclear proteins from the same alternatively spliced gene, Lamin A/C, plays a critical role in maintaining genomic stability and the nuclear genomic architecture[4,5]. Our group recently observed the upregulation of Lamin A/C expression in the MM human cell line MM.1R as well as 10 primary treatment-naïve MM patient samples. Altered 3D spatial organization was also observed for the Lamin A/C protein.

HYPOTHESIS

Downregulating Lamin A/C in Lamin A/C upregulated MM cells should return chromosomes towards their normal positions.

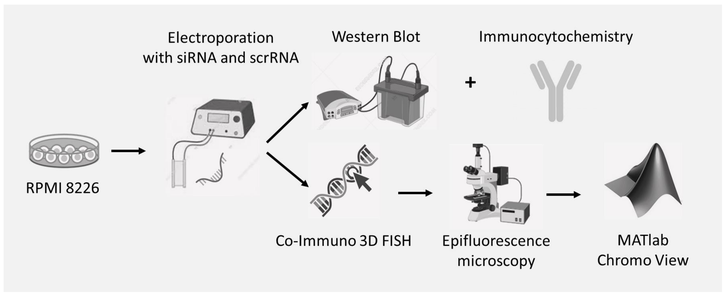
AIM

Study Lamin A/C's role on nuclear chromosomal organisation in MM.

2. Methods

Lamin A/C was downregulated in the human MM cell line RPMI 8226 using two short interfering RNAs (siRNA) that target different regions of the lamin A/C mRNA and scramble siRNA (scrRNA), through lentiviral transfection, with the purpose of evaluating Lamin A/C role in CT positions. CT changes were evaluated in chromosomes 9 and 22, which are neighbors in lymphocytes, using whole chromosome painting probes and 3D fluorescence *in situ* hybridization (FISH) followed by analysis with our published ChromoView tool[6]. Human lymphocytes and MM.1R were used as controls in the study.

Fig. 1. Summary of Experimental Workflow.



3. Results

3.1. Lamin A/C Downregulation

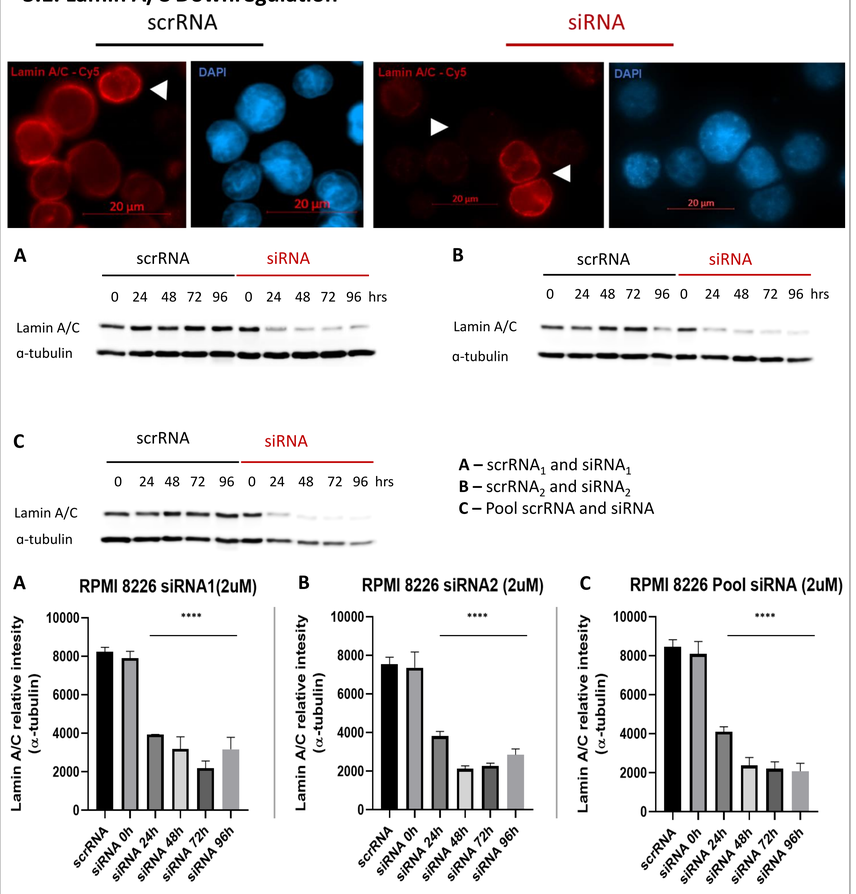


Fig. 2. Downregulation of Lamin A/C with two different siRNA. Immunocytochemistry (top) and immunoblot (middle) that shows the downregulation of Lamin A/C post siRNA transfection. Quantitative data (bottom) represented as mean ± SEM. *p*-value ≤ 0.001.

3.2. Analysis of Chromosome Terrritories

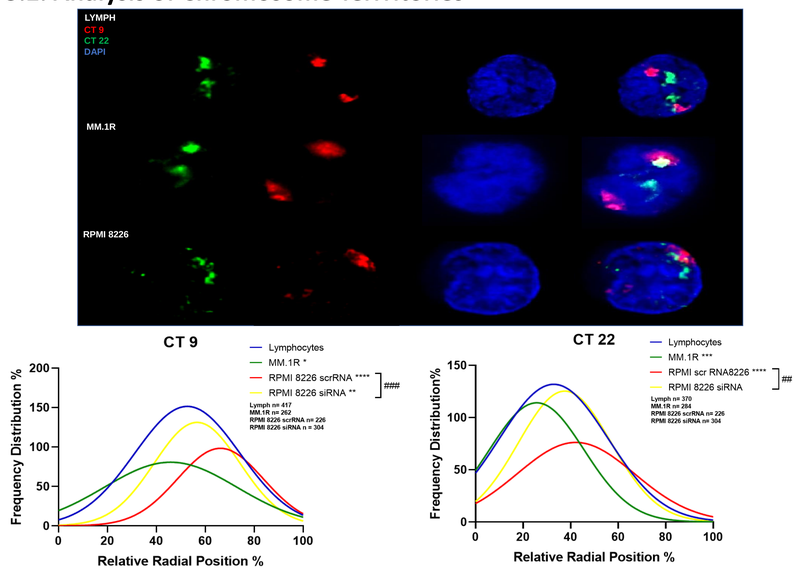


Fig. 3. Chromosome Territory Analysis 96 h after Lamin A/C Downregulation. The relative radial position of chromosomes is represented as the distance between the center of the nucleus and the center of the CT. 0% represents the nuclear center and 100% represents the nuclear periphery. Both CT9 and CT22 showed changes towards the nuclear center. Quantitative data represented as frequency of distribution. P-value ≤ 0.001.

4. Conclusion

Lamin A/C plays a known role in genomic organization and the positioning of chromosomes. As shown here, the disruption of Lamin A/C alters the CT of chromosomes 9 and 22. Cell viability of these changes should be investigated in the future, which would suggest that the disruption of lamin A/C could potentially be explored as a therapy for MM.

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