Prediction and possible molecular interactive role of wild type and HGPS mutant lamin A in connection with trf2

Niranjan Chellathurai Vasantha[1], Johnson Retnaraj Samuel Selvan Christyraj[1], Karthikeyan Subbiahnadar Chelladurai[1], Kamarajan Rajagopalan[1], Beryl Vedha Yesudhason[1], Saravanakumar Venkatachalam[1], and Jackson Durairaj Selvan Christyraj*[1]

[1]Sathyambama Institute of Science and Technology

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Abstract

Lamins are intermediate filament protein located in the inner nuclear membrane, which maintains the structural integrity and function of the nucleus. we have examined the possible interactive role of wild as well as mutant type lamin A in connection with telomere repeat binding protein trf2. Briefly, bioinformatic prediction shows that lamin A has strong interaction with telomeric complexes. DNA binding assay confirms the strong interaction between wild type lamin A and telomeric DNA sequences. Loss of 39 amino acids at C-terminal end of lamin A impairs the nuclear structural integrity and induce chromosomal fusion. We conclude that C-terminal 39 amino acids from tail domain of mature lamin A possibly interact with trf2 and telomere, not lamin C and HGPS mutant lamin A.

1 INTRODUCTION

Lamins are intermediate filaments commonly called as 'nuclear proteins' constituent of a fibrous complex, found primarily in the inner nuclear membrane labeled as nuclear lamina[1]. Apart from nuclear lamina, it is also present all over in the nucleoplasm[2,3]. In addition, lamins play an important role in maintaining shape, integrity and function of nucleus during the process of DNA replication and RNA transcription[4]. Lamin A and lamin C are isomers of lamins, derived from single LMNA gene through alternative splicing process[5]. Prelamin A contains 664 amino acid residues whereas lamin C has 572 amino acids. After post translational modifications, prelamin A converts into mature lamin A, which has 646 amino acids residues[6]. Both Lamin A and C, are expressed in all vertebrate cells and considered to be an isoforms of A-type lamins[7]. A and C type lamins were produced as a dimer throughout the rod domain[8,9]that are found to be fixed and accumulate on the exterior of mitotic chromosomes at exact places on the rod[10,11]. Lamin A interacts with a departure of nuclear factors, along with transcriptional regulators, nuclear pore complexes, nuclear membrane-associated proteins and double-stranded DNA, which disrupts lamina and protein progerin that certainly leads to modification of gene expression, and other nuclear defects that leads to genome instability[6,12].

Mutations in the lamin A leads to cause 40 different genetic diseases which includes cardio-myopathies, muscular dystrophies and Hutchinson-gilford Progeria Syndrome[12,15]. Hutchinson-gilford progeria syndrome and Werner syndrome (WS) are the two genetic diseases, well-known for the accelerated aging syndrome (Premature aging) in humans. While maximum instances of WS are due to mutation that occur in WRN helicase[16]. However, de novo G608G (GGC.GGT) mutation in exon 11 of LMNA cause approximately 90% of the peoples affected with HGPS[17,19].

Telomere in vertebrates are composed of TTAGGG repeats bounded by a protein complex "shelterin" known as telosome; this shelterin complex plays very important role in chromosome protection and also in the regulation of telomere length[20]. However, it helps in maintaining the integrity of chromosome by avoiding

unsuitable DNA destruction signaling and restoration at telomere[20,21]. At the time of dysfunctional in shelterin complex, double-stranded DNA at the chromosomal end breakdown and become subtractive for any one of six DNA-damage-response pathways, eventually it initialize to genome unstableness[21,22]. Dysfunction of telomere and DNA damage are essential characters of organismal aging and cellular senescence, these are associated with the expansion of multiple chronic inflammatory diseases, together with atherosclerosis, diabetes, and sarcopenia[23,24].

In normal cells, telomeres bind with the nuclear membrane and telomere shortening events occurred during the time of metaphase stage; Whereas, faster rate of telomere shortening events happens in lamin A mutated cells that leads to formation of chromosomal fusion. At certain stages of cell cycle, chromosomes show ring like arrangements towards nucleus[25]. Interaction between telomeres and the nuclear lamina, importantly lamin A/C play the major roles in regulation of telomere length and positioning[26]. Interaction of lamin A with telomeric complex is not studied well, rather there is lack in understanding the molecular interactions between lamin A protein with trf2 and telomere sequences. Hence, the present study was carried out to find the possible interactive role of wild as well as mutant type lamin A in connection trf2 and telomere.

2 MATERIALS AND METHODS

2.1 Prediction of lamin A-DNA and trf2- DNA interaction

DP-Bind, a web server for sequence-based prediction of DNA-binding residues in DNA-binding proteins was used in this study[39]. Human, lamin A, and trf2 protein sequences were retrieved from PubMed in FASTA format and performed for the prediction analysis. Briefly, the PSSM-based encoding was used by the DP-bind algorithm for the most accurate prediction. The results were documented and clearly showed through graphical representations using GraphPad Prism, Version 5.01.

2.2 Cell culture experiments

Human Dermal Fibroblast (HDF) cells were purchased from Thermo fisher scientific (Catalog No: C0045C), and maintained in Dulbecco's Modified Eagle's Medium (DMEM; Sigma), supplemented with 10% fetal bovine serum (FBS; Hyclone South American Origin) and 1% antibiotics (such as penicillin (100U/mL), streptomycin (100µg/mL) and amphotericin B (100µg/mL)).

2.3 Protein-DNA interaction with DNA binding assay

Genomic DNA was extracted from HDF cells using HIMEDIA kit (Cat. No: MB506-50PR) and restricted with a Rsa1 and Hinf1 restriction enzymes purchased from New England Bio Labs (Catalog number R0167S and R0155S). After digestion, telomeric repeats (TTAGGG)n were mixed with DNA coating solution purchased from Thermo scientific (Catalog number 17250) and coated to the microtiter plates. Coated microtiter plates were incubated at RT for 1-2 hours with gentle shaking. Protein lysate was prepared from HDF cells and incubated in telomeric DNA coated microtiter plates for 3 hours at 4°C. Unbound proteins were washed with 1X PBS. Primary antibodies (Lamin A (1:100) and trf2 (1:100)) in 1% BSA were prepared and added to each well. Microtiter plates were incubated overnight at 4°C. After repetitive washing with 1X TBST buffer at RT, the plates were incubated with secondary antibody (anti-rabbit IgG Alexa fluor 488 (1:1000) and anti-mouse IgG Alexa fluor 647 (1:1000)) respectively, in a dark place for 2 hours at RT. Excitation and Emission readings of the samples were taken using multi-plate reader at 488 and 647 nm, respectively.

2.4 Immunoprecipitation

Cultured HDF cells were collected and subjected to protein extraction. Briefly, RIPA buffer was added to the cells and incubated at 4°C for 10 minutes. Cells were detached by vigorous pipetting and followed by continuous rapid syringe outing. The lysate was collected through centrifugation at 12,000 rpm for 5 minutes at 4°C. To the 1 ml of protein lysate, 2µg of primary antibody (anti-trf2 antibody) was added and incubated for 2 hours at 4°C. In the mixture, 20µl of agarose beads were added to the lysate and incubated at 4°C on rocker for 2 hours. After incubation, protein lysate was centrifuged at 2,500 rpm for 5 min at 4°C. The pellet was washed three times with RIPA buffer and re-suspended in 40µl of 2X protein sample buffer and

incubated at 100 °C for 5 minutes. The immunoprecipitated protein was collected and estimated by Lowry's method and stored accordingly at -20°C for immunoblotting experiments.

2.5 SDS PAGE and Immunoblotting

Immunoprecipitated protein sample was resolved in 10% SDS-PAGE, transferred to the PVDF membrane and blocked with 4% BSA followed by incubation with primary antibody (anti-lamin A antibody). The membrane was washed with 1X TBST buffer and incubated with secondary antibody conjugated with HRP. The membrane was developed with DAB substrate and the results were documented using gel documentation system.

2.6 Immunofluorescence

Cultured HDF cells were fixed with ice-cold methanol followed by permeabilization with Triton X-100 and blocked with 1% BSA (Bovine Serum Albumin). After blocking, the cells were incubated with primary antibody (anti-lamin A and anti trf2) overnight, washed with 1X PBS and incubated with fluorophore conjugated secondary antibodies. Slides were mounted and observed under EVOS fluorescence microscope with DAPI as a counterstain.

2.7 Vector Design and transfection

Wild type GFP lamin A construct was gifted from Professor Kaushik Sengupta, Saha Institute of nuclear physics. Mutant lamin A (HG[?]50) construct was gifted from Prof. Junko Oshima from University of Washington, USA. HDF cells were transfected with mutant HG[?]50 and wild type lamin A vectors through lipid-mediated gene transfer (Lipofactomin 3000, catalog: L3000008). The transfected cells were cultured at T-flask in DMEM medium supplemented with 10% FBS under 5% CO2 at 37degC separately, and used for further experiments, accordingly.

2.8 Metaphase arrest and chromosome fusion studies

Cells were seeded in 6 well plates followed by 1 µg/ml of colcemid was added and incubated for 3 hours at 37°C with 5% CO2. Cells were collected by using trypsinization protocol, centrifuged at 1000rpm for 4 mins at RT, resuspended the pellet with 0.56% ice-cold KCL solution, mixed gently and stand for 6 min at room temperature. Drop-by-drop of 5ml fixative solution 3:1 ratio (Methanol: Acetic acid) was added to the cells. After centrifuge, 1 ml of fixative mix and small volume of mixture was released dropwise on a clean glass slide. Mounting medium with DAPI (Nuclear stain) was added to the glass slide and coverslip was placed and mount with nail polish for observation and documentation under EVOS epi-fluorescence microscope.

2.9 Lamin A and trf2 interaction by Immunostaining

Wild and mutant transfected cells were grown on a coverslip in 6 well plates. Cells were fixed with ice-cold methanol followed by permeabilization with Triton X-100 and blocked with 1% BSA (Bovine Serum Albumin). After blocking, cells were incubated with primary antibodies (anti-lamin A and anti TRF2). Coverslip was washed with 1X PBS and incubated with fluorophore conjugated secondary antibodies, counterstained with DAPI. Coverslips were mounted and observed under EVOS epi-fluorescence microscope.

2.10 Nuclear morphological study

Wild type and HGPS lamin A mutated cells were cultured on a coverslip in 6 well plate. Cells were incubated with primary anti-lamin A antibody followed by secondary antibody, anti-rabbit IgG Alexa Fluor 647. The slides were counter stained with DAPI, mounted using the mounting medium and observed under the EVOS epi-fluorescence microscope for nuclear morphological analysis.

2.11 Statistical analysis

Statistical analysis such as mean, standard deviation, standard error for the fluorescence intensity of DNA-protein binding assay was performed using statistical software - GraphPad Prism, Version 5.01.

3 RESULTS

3.1 Prediction of lamin A and telomeric DNA interaction

Human lamin A protein sequence was retrieved from the protein data bank and used for the prediction of telomeric repetitive DNA sequences $(TTAGGG)_n$ binding capability. Briefly, the bioinformatics tool namely DP bind was used for this study. The default prediction parameters were used for the analysis as suggested by the algorithm. The prediction of lamin A and telomeric DNA binding interaction was shown in (Figure 1A). The prediction clearly shows that amino acid residues of lamin A from 607 to 646 have high proximity towards telomeric repetitive DNA sequences. In addition, the algorithm scoring indicates that the tail region of lamin A was predicted as high level of interaction with telomeric DNA sequences. The known telomere repeat binding protein trf2 was selected as a positive control for DP bind analysis. Similarly, trf2 protein sequence was retrieved from protein data bank and used for the prediction of telomeric DNA binding capability. The result was shown in the (Figure 1B), which confirms that the DP bind algorithm shows accurate result, that telomeric DNA binding domain of trf2 show high proximity towards telomeric DNA sequences. Based on the preliminary data, further experiments were performed to prove the following hypothesis; 1. Interaction of lamin A with trf2 and 2. Interaction of lamin A with telomere repetitive DNA sequences (TTAGGG)n.

3.2 Interaction of lamin A with telomeric complex

In order to confirm the bioinformatics data and to prove the hypothesis, the genomic DNA was extracted from the human dermal fibroblast (HDF) cells and restricted with specific restriction enzymes namely; Rsa I and Hinf I for telomeric DNA coating experiments. Restricted genomic DNA has intact telomeric repetitive sequences (TTAGGG)n. After coating of the telomeric repetitive DNA, lamin A and trf2 specific antibodies were added into protein lysate and determined the lamin A as well as trf2 binding capability. The data was shown in the (Figure 1C), which clearly illustrates that lamin A binds strongly with the telomere repetitive DNA sequences (TTAGGG)n. Similarly, as a positive control trf2 shows high fluorescence intensity, which indicates that trf2 has strongly bound with telomere repetitive DNA. Interestingly, the fluorescence intensity of trf2 is higher than lamin A, which indicates that very short percentage of lamin A amino acid residues may interact with the telomere repetitive DNA sequences. Further to validate the data, we performed co-immunoprecipitation.

3.3 Lamin A and trf2 co-immunoprecipitation analysis

To study the interaction between lamin A with trf2, immunoprecipitation was performed. Briefly, protein lysate was prepared from human dermal fibroblast (HDF) cells and immunoprecipitated with trf2 antibodies and immunostained with lamin A antibodies. The data was shown in the (Figure 1D), which indicates that lamin A signals was clearly visualized in the membrane. This indicates that lamin A has strong interaction with trf2. Similarly, we performed co-immunoprecipitation with trf2 antibody and stained with lamin A antibody and vice versa. Results for co-immunoprecipitation was shown in the (Figure 1E), indicates the presence of lamin A and trf2 signals in the membrane, clearly shows that lamin A and trf2 have strong interaction among them. Further to validate the data, we have performed immunofluorescence assay.

3.4 Immunofluorescence analysis

Further to study the interaction of lamin A and trf2 inside the fibroblast cells, we performed immunofluorescence assay with lamin A and trf2 specific antibodies. Briefly, fibroblast cells were fixed and stained with lamin A and trf2 antibodies. Both, lamin A and trf2 signals were neatly visualized under fluorescence microscope as red and green fluorescence, respectively (data not shown). Upon close observation, it was noted that trf2 signal was closely nearest to the lamin A signal. Hence, based on the immunofluorescence data, it is confirmed that lamin A has strong interaction with trf2.

3.5 Nuclear morphometric analysis of HGPS mutant and wild type lamin A

In order to confirm that, loss of 39 amino acid residues at C terminal end of mature lamin A disrupts the structural integrity of the nucleus; we performed wildtype and HGPS mutant lamin A transfection experiments. Data for the mutant and wildtype lamin A transfection was shown in the (Figure 2). The data clearly implies that, the structural integrity of the nucleus was highly affected in HGPS mutant and noted as

irregular and lobulated nuclei. Whereas, wild type lamin A transfected fibroblast cells show circular nuclei. This data confirms that, loss of 39 amino acid residues at C terminal end of mutant lamin A causes the disruption of nuclear structural integrity.

3.6 Wild type and HGPS mutant lamin A analysis

Lamin A is a type V intermediate filament family protein, which is present in the inner nuclear membrane of the nucleus. Human premature lamin A has totally 664 amino acid residues, whereas mature lamin A has 646 amino acid residues, which forms functional lamin A and gives structural integrity to nucleus and perform vital role in many signaling pathways including interactions with telomeric protein complex. The schematic representation of wild as well as HGPS mutant lamin A and lamin C was shown in the (Figure 3) which clearly illustrates the structural abnormalities between lamin A with missing of 39 amino acid residues in HGPS mutant.

3.7 Immunoblotting and chromosomal fusion analysis

To validate the wildtype and HGPS mutant lamin A transfection we performed immunoblotting. Briefly protein lysate was prepared and stained with lamin A antibodies, results for immunoblotting was shown in the (Figure 4A). Results clearly shows that, in wildtype lamin A transfected cells, single prominent band signal was visualized confirms lamin A. Rather, protein samples from HGPS mutant lamin A transfection cells, two closely related signal visualized and marked as red color arrow in the (Figure 4A). The difference between mature lamin A and HGPS mutant lamin A is only 39 amino acids. Hence, two close signals were visualized in lane 3 and 4 of (Figure 4A). To confirm the data, we analyzed the blot with gel analyzer software, which clearly shows the presence of single band in wild type and double band in HGPS mutant cells. Results for the gel analysis is shown in the (Figure 4B). Black and Red asterisk indicates wild type and HGPS mutant lamin A, respectively. Further to study the chromosomal fusions in HGPS mutant as well as wild type lamin A transfected HDF cells, we performed metaphase arrest after the transfection. Results for metaphase arrest for chromosome fusion analysis was shown in the (Figure 4C, D). The data clearly shows that, chromosomal fusion was noted in the HGPS mutant and not in the wild type and control lamin A transfected cells. In addition, it is clear that, HGPS mutation causes loss of 39 amino acid residues at the C-terminal end of lamin A, which induce chromosomal fusion.

4 DISCUSSION

In the present study, we have predicted the possible interaction of lamin A with trf2 and telomeric repeats, thereby contribute in the structural integrity of the nucleus. First, we studied in detail about the wild type and mutant lamin A and its phenotypes. Based on the literature, we hypothesized that wild type lamin A has an interaction with telomeric repetitive sequences (TTAGGG)n. Hence, we performed bioinformatics analysis to prove our hypothesis. Our data suggest that the DP-bind simulation-based bioinformatics approach confirms that lamin A has strong interaction with telomere repeats (TTAGGG)n in addition to trf2. To support the claim, we performed similar analysis with trf2 protein, which has 542 amino acid residues[27]. Previous reports clearly suggested that trf2 has strong interaction with telomeric repeats[28,29]. Specifically, trf2 has DNA binding domain at 484 to 541 amino acids. We have chosen trf2 as a positive control for DP-bind bioinformatic tool to study the lamin A – DNA interaction. Our data confirms that lamin A has strong interaction with telomeric repeats.

To validate the bioinformatic data, we performed experiments to check the lamin A and telomere interaction using DNA binding assay. According to the report, DNA coating solution was used to anti-dsDNA measurement for calf thymus DNA[30]. Our data suggest that lamin A binds specifically to repetitive telomere DNA sequences. Similarly, as a positive control, trf2 shows signal, which illustrates that trf2 has capable of binding with telomere region[31].

Interestingly, lamin A not only binds to telomeric repeats rather, it interacts with trf2 of telomeric complex called as 'shelterin'. To confirm the data, we performed co-immunoprecipitation assay with lamin A and trf2 antibodies, vice versa. Our data confirms that protein lysate immunoprecipitated with lamin A antibody

shows signal for both lamin A as well as trf2. Similarly, protein lysate immunoprecipitated with trf2 antibody shows signal for both trf2 and lamin A. The data clearly indicates that lamin A and trf2 has closely interacted with each other in the shelterin complex**.** Lamin C is the splicing variant of lamin A[32], which has only 572 amino acid residues and it lacks carboxyl terminal domain of last 98 amino acid residues of mature lamin A. Our results show that co-immunoprecipitation of wild type lamin A with trf2 and vice versa confirms that lamin A binds with trf2 and not lamin C. Because as mentioned earlier, lamin C lacks the carboxyl terminal domain of 98 amino acid residues of mature lamin A. Based on the observation, it is clear that tail domain of lamin A plays a crucial role in binding with shelterin complex.

Based on the data, lamin A binds with both trf2 and telomeric DNA repeats, but specifically it is not known that whether lamin A binds to minor or major groove of the telomeric repetitive sequences (TTAGGG)n. Interestingly, it is known that trf2 specifically binds with telomeric repetitive DNA sequences at major as well as minor groove[33]. Hence, it may be a chance that lamin A binds at major groove of telomeric DNA repetitive sequences. Because, N-terminal region of trf2 binds to minor groove of telomeric repetitive DNA sequences[33]. At specific stage of cell cycle, the chromosome arrangement looks like a ring structure, which is due to the interaction of lamin A with trf2 and telomere[25]. We have performed experiments and our results conclude that lamin A binds with trf2 rather; it is not known whether lamin A binds with any of the sheltron complex proteins.

Fusion, degradation, recombination in chromosomes can be protected specifically by telomere repeats[34]. Telomere repeats are generated by telomerase reverse transcriptase (TERT), which recognizes chromosome ends and synthesizes telomere repeats via de novo using a telomerase-associated RNA molecule (TERC) as a template[35]. But in the case of HGPS mutant lamin A, premature aging occurs frequently suggest that lamin A has interactive role with telomere. Bioinformatic data confirms that wild-type lamin A, especially C-terminal end of 39 amino acid residues have strong interaction with telomere region and thereby holds the aging process. But, in the case of HGPS mutant lamin A, 39 amino acids residues were not present and hence premature aging begins frequently. The complete mechanism behind the premature aging is not clearly studied.

Loss of 39 amino acid residues in HGPS mutant lamin A not only cause premature aging, but also impairs the nuclear structural integrity and induce chromosomal fusion. The reason for chromosome fusion might be due to; 1. Mutation in the lamin A results in loss of C terminal 39 amino acid residues and hence, it is not specifically binding with telomeric complexes (specifically trf2 and telomeric repetitive sequences (TTAGGG)n); and 2. Loss of C terminal 39 amino acid residues in HGPS mutant lamin A results in impairing in the nuclear structural integrity.

Transfection of wild type and HGPS mutant lamin A into fibroblast cells shows that, compare to control cell, mutated cell nuclear morphology was impaired with irregular lobulated nuclei. Loss of C terminal 39 amino acid residues of lamin A results in structural deformities of the nucleus. Reason for the nuclear structural impair might be due to; 1. Loss of C terminal 39 amino acid residues of lamin A; and 2. Disturb in the tail domain of lamin A has impact in coiling process of forming nuclear lamina along with lamin C. Interestingly, E145K progeria patient fibroblast cells morphology shows lobulated nucleus[36]. LMNAp.R388P mutation patient cells show specific deficiencies in lamina organization and cell explosion ability[37]. Similarly, fibroblast cells overexpressing R133L and L140R mutant lamin A displayed numerous degrees of asymmetrical nuclei [38]. Based on the above reports, it is clear that any mutation in lamin A results in irregular lobulated or asymmetrical nuclei, not specifically, loss of C terminal 39 amino acid residues of HGPS mutant lamin A. Based on the data, we conclude that C-terminal 39 amino acids from tail domain of mature lamin A possibly interact with trf2 and telomere, not lamin C and HGPS mutant lamin A.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in any part of the manuscript.

AUTHORS CONTRIBUTIONS

The research work was designed by the corresponding authors, Dr. S Johnson Retnaraj Samuel and Dr. S Jackson Durairaj. The manuscript was written and communicated by the corresponding authors with the help from Dr. Y Beryl Vedha. The research work was executed by Mr. CV Niranjan and Dr. S Johnson Retnaraj Samuel. Authors – Mr. SC Karthikeyan and Mr. R Kamarajan contributed by performing animal cell culture experiments. Mr. V Saravanakumar performed restriction digestion and coating of DNA to the micro-titre plates. Bioinformatics data was generated by Dr. S Johnson Retnaraj Samuel. Statistical analysis was performed by Dr. Y Beryl Vedha. English language in the manuscript was corrected by both corresponding authors Dr. S Johnson Retnaraj Samuel and Dr. S Jackson Durairaj.

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Figures Legend:

FIGURE 1 Interaction of lamin A and trf2 in the connection with telomere. A, Bioinformatic approach of finding the interaction between lamin A and telomeric repetitive sequences. Represents interaction between Lamin A (from 607 to 646 amino acids) with telomere DNA using DP-Bind bioinformatics tool. B, Represents interaction between trf2 (from 509 to 542 amino acids) with telomere DNA using DP-Bind bioinformatics tool. C, DNA-Protein interaction assay. Represents fluorescent intensity of Lamin A and trf2 interaction with telomeric repetitive DNA sequences using DNA binding assay. D, Immunoprecipitation assay. Lane 1 – Protein ladder; Lane 2 to 8 – immunoprecipitated samples stained with anti-lamin A antibodies. E, Western blot validation of trf2 and Lamin A co-immunoprecipitation with anti-Lamin A and anti-trf2 antibodies. Lane 1, co-immunoprecipitation of Lamin A protein with anti-Lamin A antibody. Lane 2, co-immunoprecipitation of Lamin A protein with anti-trf2 antibody.

FIGURE 2 Immunofluorescence assay and nuclear structural integrity of wild type and HGPS mutant lamin A. Cells were stained with anti-lamin A antibody and counter stained with DAPI. Arrow mark indicates the folding and wrinkles in nuclear membrane.

FIGURE 3 Schematic representation and overview of wild type / HGPS mutant lamin A and trf2 interaction in nuclear structural integrity.

FIGURE 4 Transfection of wildtype and mutant lamin A and functional studies. A, Immunoblotting analysis of wildtype and HGPS mutant lamin A transfected fibroblast cells stained with anti-lamin A antibodies, Lane 1 – Protein marker, Lane 2 – Wild type lamin A transfected sample, Lane 3 & 4 – HGPS mutant lamin A transfected sample. Black arrow mark indicates wild type lamin A, whereas red arrow mark indicates HGPS mutant lamin A. B, Analysis of wild type and HGPS mutant lamin A blot using Gel analyzer software. Black and red asterisk indicates wild type and HGPS mutant lamin A, respectively. C, Chromosomal fusion assay – Control. D, Chromosomal fusion analysis of HGPS lamin A mutant.