Nurturing the genome

A-type lamins preserve genomic stability

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A-type lamins provide a scaffold for tethering chromatin and protein complexes regulating nuclear structure and function. Interest in lamins increased after mutations in the *LMNA* gene were found to be associated with a variety of human disorders termed laminopathies. These include muscular dystrophy, cardiomyopathy, lipodystrophy, peripheral neuropathy and premature aging syndromes such as progeria. In addition, altered expression of A-type lamins is emerging as a contributing factor to tumorigenesis. How different alterations in a gene that is ubiquitously expressed can cause such an array of systemic as well as tissue specific diseases remains an enigma. Several lines of evidence indicate that mutant forms of A-type lamins impact on genome function and integrity. A current model suggests that genomic instability plays a major part in the pathophysiology of some lamin-related diseases. However, this model remains to be fully investigated. Here we discuss recent studies revealing novel functions for A-type lamins in the maintenance of telomeres and in the DNA damage response (DDR) pathway. These findings have shed some light onto the putative molecular mechanisms by which alterations in A-type lamins induce genomic instability and contribute to disease.

Introduction

Recent studies suggest that defects in the ability of cells to properly repair DNA damage contribute to the genomic instability of some laminopathies, especially premature aging diseases such as Hutchinson Gilford Progeria Syndrome (HGPS). Our findings demonstrate that the loss of A-type lamins in mouse cells leads to alterations of telomere structure, length and function, in addition to defects in the DDR pathway. These results suggest that deficiency in A-type lamins could contribute to the genomic instability that drives tumorigenesis and premature aging phenotypes. At the molecular level, loss of A-type lamins leads to the destabilization of 53BP1 protein, a key mediator in DDR, thus providing a putative mechanism by which loss of A-type lamins induces genomic instability.

A-type lamins. Lamins A and C, members of the type V intermediate filaments family,[1] are main components of the nuclear lamina[2] and are also found as part of a nucleoplasmic network that associates tightly with chromatin.[3-5] A-type lamins are primarily expressed in cells after the onset of differentiation[6] and are thought to exert specialized functions.[7] Synthesis of Lamin A entails unique posttranslational processing events, converting a prelamin A precursor into the mature lamin A form. This processing includes farnesylation of prelamin A carboxy-terminal CAAX motif, cleavage of—AAX, carboxymethylation of the terminal cysteine, and a second cleavage of the last 15 amino acids by the metalloprotease Zmpste24.[8-10] A mutation in the *LMNA* gene that abrogates the second cleavage site leads to the expression of a dominant-negative prelamin A isoform known as progerin, which causes HGPS, the most

Table 1. Comparison of the most relevant phenotypes present in cells from *Lmna* K.O. and *Zmpste24* K.O. mice and HGPS patients

		Lmna K.O.	*Zmpste24* K.O.	HGPS
Lifespan		8 weeks[53]	20 weeks[10]	12.6 years[13]
Nucleus	**Morphology**	Aberrant[53]	Aberrant[10]	#Aberrant[11,12]
	Chromatin	Detachment from periphery[53]	Aggregates[10,14,40]	Detachment from periphery[54]
Telomere	**Length**	Shortening[59]	No change[10]	Shortening[48,49]
	Localization	Nucl. periphery[59]	-	-
Heterochromatin	**H3K9me3**	No changes[59]	-	#Decreased[42,73]
	HP1	-	-	#Decreased[73]
	H4K20me3	Decreased[59]	-	Increased[73]
	TERRAS	Decreased[59]	-	-
Genomic instability	**STLs**	Increased[59]	-	Increased[49]
	Chrom. breaks	Increased[59]	Increased[40]	-
	Aneuploidy	Yes[59]	Yes[40]	-
DNA damage response	**γ-H2AX (Basal)**	Increased[59]	Increased[11,40]	#Increased[40,42]
	53BP1	Decreased levels[59]	Increased basal foci[40]	Increased basal foci
	DNA repair	Defective NHEJ[59]	Defective HR[40]	Defective HR[40]
p53		-	Hyperactivated[14]	Hyperactivated[42]
Retinoblastoma		Decreased[55,56,59]	Decreased[10]	Present[56,58]

#Also in fibroblast from old individuals.[42]

severe laminopathy.[11-13] Accordingly, mice knockout for Zmpste24 exhibit similar phenotypes as human patients with HGPS.[10,14] Hundreds of disease-associated mutations have been identified within the *LMNA* gene.[7,15,16] In addition, changes in the expression of lamins are observed in leukemia, lymphomas, small cell lung and ovarian cancer, as well as colon carcinoma. Interestingly, these changes are often associated with increased aggressiveness and poor prognosis.[17-20] The broad range of diseases associated with either mutations in the *LMNA* gene or changes in the expression of A-type lamins has recently attracted much attention towards elucidating the functions of these structural nuclear proteins.

Telomere structure, length and function. Alterations in telomere function are a hallmark of cancer and aging phenotypes. Proper telomere function relies on the formation of a specialized higher-order structure that shelters the ends of linear chromosomes from the attack of nuclear activities, preserving chromosome integrity and cellular proliferative potential.[21-24] Formation of this structure requires a minimal length of telomeric DNA repeats and a number of telomere structural proteins. These include a multiprotein complex known as "shelterin" or "telosome" and a number of DNA repair factors.[25-28] Telomeric DNA is maintained primarily by telomerase[29] or by the Alternative Lengthening of Telomeres (ALT) mechanism.[30,31] Telomeric chromatin modulating activities such as histone methyltransferases,[32-34] Rb family members[35,36] and DNA methyltransferases[37] also play a role in the maintenance of telomere length homeostasis.[38] In summary, the action of telomere length maintenance mechanisms, telomere binding proteins and telomere chromatin-modifying activities ensures the preservation of telomere length homeostasis and the end-capping function of telomeres.

Role for A-Type Lamins in the DDR

Mutations in the *LMNA* gene can induce genomic instability. Common defects in the DDR have been observed in the premature aging laminopathies HGPS and Mandibuloacral dysplasia type A (MADA) and in the *Zmpste24*-/- mouse model of progeria (**Table 1**). Fibroblasts from patients with MADA, caused by the homozygous R527H mutation in the *LMNA* gene, are impaired in their ability to repair DNA damage. This is demonstrated by reduced activation of p53 and its downstream targets, increased chromosome damage and residual γH2AX foci, which are characteristic of unrepaired DNA lesions.[39] Fibroblasts from HGPS patients and *Zmpste24*-/- mice have defective DNA repair pathways and increased basal DNA damage levels, exhibiting chromosome aberrations and increased sensitivity to DNA-damaging agents.[14,40] These cells also show delayed recruitment of 53BP1 to γH2AX-labeled DNA repair foci after ionizing radiation and delayed resolution of these foci. Furthermore, progeroid fibroblasts exhibit impaired recruitment of double-strand break (DSB) repair factors Rad50 and Rad51 to sites of DNA damage, as well as aberrant accumulation of Xeroderma Pigmentosum group A (XPA) protein, which is in part responsible for the defects in DNA repair.[40,41] Additional studies showed that HGPS fibroblasts display an activated DDR, as manifested by enhanced basal γH2AX and active p53.[42] Overall, these studies provide a strong correlation between proper processing of lamin A and the maintenance of genomic stability. In addition, they indicate that an altered ability to properly repair DNA damage could contribute to the pathophysiology of premature aging laminopathies.

Role for A-Type Lamins in Telomere Biology

Binding of lamins A/C to telomeric sequences in vitro was first reported in 1988 by Shoeman et al.[43] Subsequent studies revealed that human telomeres associate with the nuclear matrix[44] putatively through telomere binding proteins.[45] More recent studies have confirmed the binding of A-type lamins to mammalian telomeres in vivo by chromatin immunoprecipitation (ChIP).[46] Using live cell imaging and fluorescence microscopy, the binding of lamina-associated proteins such as LAP2α and BAF to telomeres during nuclear assembly was also demonstrated.[47] This suggests a putative role for these proteins in mediating the tethering of telomeres to A-type lamins. The extent of this tethering and the functional implications for telomere biology are only beginning to be uncovered.

The best evidence for a role of A-type lamins in telomere biology comes from HGPS fibroblasts, which exhibit faster telomere attrition than normal counterparts.[48] These results were confirmed by a study that monitored the length of individual telomeres in fibroblasts and hematopoietic cells from HGPS patients[49] (**Table 1**). Interestingly, fibroblasts expressing progerin display telomere shortening and an increase in signal free ends while hematopoietic cells, which do not express detectable levels of lamin A, display normal telomere length. The authors concluded that the expression of progerin is necessary for the telomere shortening phenotype, and that the effect of A-type lamins on telomere length is direct. In clear contrast to these results, alterations of telomere length have not been observed in Zmpste24^{-/-} fibroblasts[10] (and our unpublished results). This discrepancy might be due to differences in telomerase activity between mouse and human somatic cells. Despite normal maintenance of telomere length, it is possible that Zmpste24 deficiency induces telomere dysfunction. In another study, isogenic lines of skin fibroblasts were transfected with wild-type or mutant forms of lamin A previously associated with progeroid syndromes. Nuclear abnormalities, a faster rate of telomere attrition, and a shortened replicative lifespan

were observed upon expression of either the wild-type protein or the mutants.[50] These results suggest that mutations in the *LMNA* gene and changes in the expression of A-type lamins can affect telomere homeostasis. Further evidence of crosstalk between A-type lamins and telomeres is the finding that the proliferative defects of human fibroblasts expressing lamin A mutants are rescued by telomerase.[51] In spite of the data indicating that A-type lamins play a role in the maintenance of telomeres the molecular mechanisms remain unknown. Similarly, whether or not alterations in telomere biology contribute to the phenotype of lamin-related diseases need to be determined.

Genomic Instability in Mouse Models of Laminopathies

Mouse models of laminopathies have become instrumental in the understanding of the molecular basis of these diseases.[52] The *Lmna^{-/-}* and *Zmpste24^{-/-}* mouse models have been the most extensively characterized[10,14,53] (**Table 1**). At the cellular level, both models recapitulate some HGPS phenotypes such as ultrastructural defects of the nuclear envelope and loss of heterochromatin from the nuclear periphery.[10,53,54] *Zmpste24^{-/-}* fibroblasts also feature defects in DNA repair;[40] observation that led investigators to propose that increased genomic instability contributes to the pathogenesis of progeria. The phenotype of *Lmna^{-/-}* cells with respect to genomic instability is yet to be fully characterized. Molecular studies suggest that A-type lamins exert a role in tumor suppression. Loss of A-type lamins induces destabilization of Rb family members[55,56] and reduced levels and mislocalization of ING1,[57] which are proteins with well-established tumor suppressor functions. In the case of Rb family members, A-type lamins deficiency promotes their degradation by the proteasome, rendering cells unresponsive to Rb-mediated signals.[56] Hence, *Lmna^{-/-}* fibroblasts exhibit similar characteristics to Rb-deficient cells, including increased pool of proliferating cells undergoing DNA replication, premature entry of cells into S-phase after release from a G_1 arrest, smaller cellular size, and failure to undergo growth arrest

in response to DNA damage.[55] The status of the Rb pathway in fibroblasts from HGPS patients is controversial. A recent study showed decreased Rb levels in lines of HGPS patients.[57] However, others have reported normal levels of Rb but a marked decrease in the inactive hyperphosphorylated form of the protein required for the G_1 to S transition of the cell cycle.[58] Accordingly, reconstitution of mutant forms of lamin A associated with HGPS into *Lmna^{-/-}* fibroblasts increased the cellular levels of Rb and rescued the ability of these cells to respond to Rb-mediated signals.[56] Based on these data, it is tempting to speculate that expression of progerin increases the pool of active Rb, limiting the proliferative potential of HGPS cells. In contrast, loss of A-type lamins and reduced Rb levels would contribute to the uncontrolled proliferation that characterizes tumor cells. Consistent with this model, a variety of human tumors silence the *LMNA* gene via hypermethylation of its promoter.[17-20] In most cases, downregulation of A-type lamins was associated with increased tumor aggressiveness and poor prognosis.

Our recent data indicate that complete loss of A-type lamins also leads to genomic instability. In particular, alterations of telomere structure, length and function, and destabilization of a key factor (53BP1) in the DDR was observed in *Lmna^{-/-}* mouse fibroblasts (**Table 1**). These results suggest that the loss of A-type lamins, which characterizes certain human tumors, could contribute to the genomic instability that drives malignancy. Future studies are needed to address if the loss of A-type lamins in human cells recapitulates the defects in telomere biology, DNA repair and overall genomic instability reported for mouse cells.

Alterations of Telomere Metabolism in the *Lmna^{-/-}* Mouse Model

Loss of A-type lamins induces a modest telomere shortening phenotype and a pronounced increased frequency of signal-free ends (loss of telomeric signals)[59] (**Fig. 1 and Table 1**). The fact that telomere shortening is rescued by reconstitution of either lamin A or lamin C indicates that
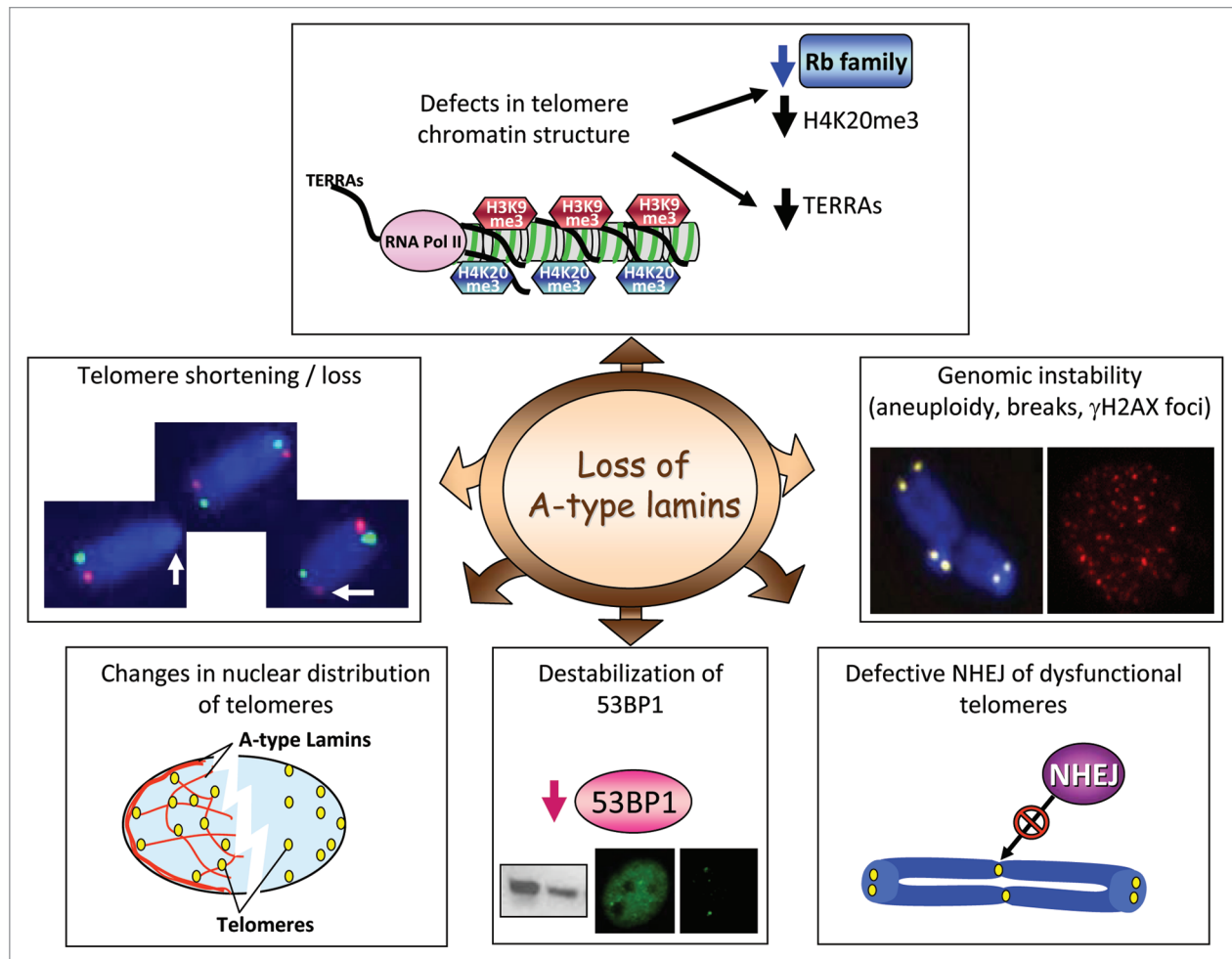
Figure 1. Loss of A-type lamins induces genomic instability. Summary of the recently identified alterations in telomere biology and in the DNA damage response pathway upon loss of A-type lamins in mouse cells.[5,9] *Lmna⁻/⁻* fibroblasts exhibit alterations in the structure of telomere chromatin Specifically, a decrease in H4K20me3 and TERRAs levels was observed. The decrease in H4K20me3 is consistent with destabilization of Rb family members in these cells. In addition, loss of A-type lamins leads to alterations in the nuclear distribution of telomeres as determined by 3D FISH, and in telomere metabolism. Telomere shortening—as shown by TRF and Q-FISH assays—and telomere dysfunction—increased number of chromosomes that feature undetectable telomere tracks—were observed. Furthermore, *Lmna⁻/⁻* fibroblasts feature aneuploidy, chromosome and chromatid breaks, and basal γH2AX foci indicative of unrepaired DNA damage. Loss of A-type lamins also hinders the processing of dysfunctional telomeres by NHEJ. The concomitant destabilization of 53BP1 protein could be in part responsible for the increased genomic instability and the defects in NHEJ upon loss of A-type lamins. Collectively, these data indicate that A-type lamins function in the maintenance of genome stability.

both forms of A-type lamins participate in telomere length control.[60] An in vitro assay showed no differences in the overall telomerase activity between *Lmna⁺/⁺* and *Lmna⁻/⁻* fibroblasts. In addition, similar telomere-bound levels of shelterin complex components TRF1 and TRF2 were found in both genotypes.[59] These results suggest that an alternative mechanism is responsible for the telomere shortening/loss in *Lmna⁻/⁻* cells.

Expression of lamin A mutants has been shown to impact DNA replication and repair.[61,62] In addition, a study demonstrating that proliferative defects

of human fibroblasts expressing lamin A mutants are rescued by telomerase[51] suggests that telomerase exerts a protective function against telomere defects induced by alterations in A-type lamins. This protective function of telomerase has also been shown in cells deficient in other factors participating in telomere replication and repair, such as the RecQ helicase Werner[63] and the flap endonuclease FEN1.[64] Replication of telomeres presents unique problems due to the linear nature of chromosomal DNA and the formation of unusual structures (G-quadruplexes and T-loops) that slow or even stall fork

progression. Recruitment of factors involved in DNA repair/recombination to telomeres is thought to facilitate the repair of DNA lesions that occur during telomere replication, as well as contribute to the formation of the higher-order structure of telomeres after completion of replication.[28] Telomere replication and telomerase-dependent telomere elongation are highly coordinated processes. They require proper functioning of the DNA replication machinery, binding of the shelterin complex components and DNA repair factors, and the accessibility of telomerase. Alterations in any of these processes can

impact telomere metabolism. We speculate that the loss of A-type lamins affects the proficient coordination of telomere replication/repair and telomerase-dependent telomere elongation and that the overexpression of telomerase overcomes some of these defects.

Alternatively or concomitantly, aberrant recombination events involving telomeres could contribute to the telomere shortening/loss phenotype upon loss of A-type lamins. Three types of recombination events involving telomeres have been described: (1) telomeric sister chromatid exchanges (T-SCE) which often result in the elongation of one telomere at the expense of shortening the sister telomere and are characteristic of ALT-positive tumor cells;[65] (2) formation of telomeric circles (T-circles)[66] that result from recombination at the level of the T-loop; and (3) formation of telomeric DNA-containing double minute chromosomes (TDMs), which are extrachromosomal elements resulting from either recombination of telomeres with interstitial telomere-related sequences[67] or DSBs at fragile sites.[68] Chromosome Orientation FISH (CO-FISH)[69] showed no evidence of increased T-SCE events in *Lmna*^{-/-} cells with respect to wild-type controls.[59] However, we cannot rule out that loss of A-type lamins induces other recombination events which contribute to telomere instability.

Several lines of evidence indicate that the acquisition of a heterochromatic structure at telomeres is also critical for the maintenance of telomere length homeostasis. Trimethylation of histone H3 at lysine 9 (H3K9me3) and histone H4 at lysine 20 (H4K20me3) are characteristic heterochromatic marks of telomeres.[38] In addition, non-coding RNAs (TERRAs) accumulate at mammalian telomeres.[70,71] Our studies demonstrated that loss of A-type lamins impacts telomeric chromatin structure[59] (**Fig. 1 and Table 1**). Specifically, a decrease in H4K20me3 and TERRAs levels was observed in *Lmna*^{-/-} fibroblasts. Previous studies demonstrated that Rb family members and histone methyltransferases Suv4-20 h1/h2 stabilize the levels of H4K20me3.[32,36,72] Mouse embryonic fibroblasts triple knockout for Rb family members or double knockout

for Suv4-20 h1/h2 exhibit reduced levels of H4K20me3 at telomeric and pericentric heterochomatin. Thus, the decrease in H4K20me3 levels in *Lmna*^{-/-} fibroblasts at these domains is consistent with destabilization of Rb family members. Intriguingly, loss of Rb family members and decreased levels of H4K20me3 were previously associated with telomere elongation.[32,35] In *Lmna*^{-/-} fibroblasts however, the combined loss of A-type lamins and Rb family members and the concomitant decrease in H4K20me3 levels leads to a telomere shortening phenotype. These results suggest that A-type lamins might be necessary for the elongation of telomeres upon Rb- and H4K20me3-deficiency. Future studies are needed to identify the mechanism by which loss of Rb function leads to telomere elongation and determine whether or not A-type lamins deficiency hinders this process. An interesting observation that came out of these studies is that the defects in heterochromatic marks upon loss of A-type lamins are different from those observed upon expression of progerin in HGPS fibroblasts. A decrease in H3K9me3 and an increase in H4K20me was observed in these cells, demonstrating distinct functional consequences of complete loss of A-type lamins and expression of lamin A mutants, with respect to epigenetic defects.[59,73] Overall, our study demonstrates that complete loss of A-type lamins impacts telomere metabolism, although the specific mechanisms remain unknown.

A-type lamins have been ascribed a major role in the tethering of chromatin to specific subnuclear compartments. We found that mouse telomeres associate with A-type lamins and that loss of A-type lamins leads to a shift of telomere distribution towards the nuclear periphery and away from the nuclear center[59] (**Fig. 1 and Table 1**). In addition, a recent study demonstrated that the D4Z4 human subtelomeric repeat localizes the nearby telomere to the nuclear periphery in an A-type lamins-dependent manner.[74] Overall, these studies indicate that A-type lamins aid in the recruitment of specific sequences to the nuclear periphery as well as participate in the distribution of telomeres throughout the nucleoplasm. The functional consequences of the loss of tethering of

telomeres to A-type lamins remain to be characterized.

Defects in DDR and DNA Repair in the *Lmna*^{-/-} Mouse Model

Lmna^{-/-} fibroblasts exhibit aneuploidy, increased frequency of chromosome and chromatid breaks, increased basal levels of DNA damage, and defects in the non-homologous end-joining (NHEJ) of dysfunctional telomeres[59] (**Fig. 1 and Table 1**). Collectively, these data indicate that loss of A-type lamins hinders some steps during the sensing, signaling or repair of DNA damage. Monitoring of the levels of different DDR factors revealed a marked decrease in the levels of 53BP1 protein. 53BP1 is a key mediator in the cellular response to DNA damage, proposed to function at the interface of DNA replication, recombination, and repair. In contrast, the levels of other proteins involved in DDR and DNA repair—ATM, DNA-PK, Mre11, Nbs1, Ku70, RAD51, MDC1 and ERCC1—were not altered, implying a preferential effect on 53BP1. Importantly, reconstitution of either lamin A or lamin C rescued the levels of 53BP1, supporting a role for these proteins in the stabilization of 53BP1. Similarly, treatment of *Lmna*^{+/+} and *Lmna*^{-/-} fibroblasts with a proteasome inhibitor stabilized 53BP1 levels, implicating the proteasome in the degradation of 53BP1. Given the known role of A-type lamins in preventing the degradation of Rb family members by the proteasome,[55,56] we envision that the stabilization of 53BP1 and Rb proteins by A-type lamins might be achieved by a similar mechanism. The decrease in 53BP1 could explain some of the phenotypes observed upon loss of A-type lamins. A recent study demonstrated that loss of 53BP1 hinders the processing of dysfunctional telomeres by NHEJ.[75] The authors showed that the loss of 53BP1 restricts the mobility of dysfunctional telomeres. This led to the proposal that 53BP1 has an active role in chromatin dynamics that facilitate the association and fusion of dysfunctional telomeres that might be far away within the nucleus. 53BP1 deficiency provides a putative mechanism by which alteration of A-type lamins function impacts on this process. However, a clear demonstration is pending.

The defects in NHEJ of dysfunctional telomeres in *Lmna* fibroblasts could be solely due to destabilization of 53BP1. Alternatively, A-type lamins could play an active role in the 53BP1-mediated regulation of mobility and NHEJ of dysfunctional telomeres, other than stabilization of 53BP1. A functional relationship between 53BP1 and A-type lamins during the processing of dysfunctional telomeres requires further investigation.

53BP1 knockout mice exhibit a phenotype consistent with defects in DNA repair, such as increased radiosensitivity, immunodeficiency and cancer susceptibility. Several lines of evidence indicate the participation of 53BP1 in NHEJ of DNA double-strand breaks at internal sites of chromosomes.[76-78] Putative roles for A-type lamins in these processes await future characterization.

A role for 53BP1 in DNA replication has also been described. 53BP1 is required for efficient accumulation of the Bloom (BLM) helicase and p53 at sites of stalled replication.[79] Additional studies have shown that 53BP1, BLM and RAD51 interact during stalled replication and that loss of 53BP1 decreases cell survival and enhances chromosomal aberrations upon replication arrest.[80] We envision that the decrease in 53BP1 upon loss of A-type lamins could affect telomere replication, contributing to the defects in telomere metabolism observed in *Lmna* fibroblasts.

Summary

Previous studies showed that cells from premature aging laminopathies are hindered in their ability to properly deal with DNA damage, leading to genomic instability. Our studies revealed that complete loss of A-type lamins also affects the cellular response to DNA damage as well as the maintenance of telomere stability, which could represent a second source for genomic instability in laminopathies. In addition, our data suggest that 53BP1 deficiency may be a key factor in the genomic instability observed in *Lmna* cells. Future studies will need to determine if 53BP1 deficiency and alterations of telomere biology are phenocopied in human laminopathies and if they contribute to the pathophysiology of these diseases. In addition, determining the molecular mechanisms by which the loss of A-type lamins impacts on the different mechanisms regulating telomere metabolism and DNA repair will be fundamental for the development of novel therapeutic strategies to treat these diseases.

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