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LMNA mutations resulting in lipodystrophy and HIV protease inhibitors trigger vascular smooth muscle cell senescence and calcification: Role of ZMPSTE24 downregulation

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ABSTRACT

Background: Some *LMNA* mutations responsible for lipodystrophies, and some HIV-protease inhibitors (PIs) induce accumulation of farnesylated prelamin A and premature senescence in some cell types. Patients with *LMNA* mutations or under PI-based therapy suffer from early atherosclerosis. The metalloprotease ZMPSTE24 is the key enzyme in prelamin A maturation.
Aim: We studied whether altered expression of ZMPSTE24 could contribute to vascular cell dysfunction in response to *LMNA* mutations or PI treatments.
Methods: Protein expression of prelamin A and ZMPSTE24 were evaluated in patients' cells and in human cultured VSMCs. Oxidative stress, inflammation, senescence and transdifferentiation/calcification were evaluated in VSMCs.
Results: Fibroblasts from *LMNA*-mutated lipodystrophic patients (mutations R482W, D47Y or R133L) and peripheral blood mononuclear cells from PI-treated-HIV-infected patients expressed increased prelamin A and decreased ZMPSTE24, which was also observed in VSMCs overexpressing mutant *LMNA* or treated with PIs. These alterations correlated with oxidative stress, inflammation, senescence and calcification (all $p < 0.05$). ZMPSTE24 silencing in native VSMCs recapitulated the mutant *LMNA*- and PI-induced accumulation of farnesylated prelamin A, oxidative stress, inflammation, senescence and calcification. A negative regulator of ZMPSTE24, miRNA-141-3p, was enhanced in *LMNA*-mutated or PI-treated VSMCs. The farnesylation inhibitors pravastatin and FTI-277, or the antioxidant N-acetyl cysteine, partly restored ZMPSTE24 expression, and concomitantly decreased oxidative stress, inflammation, senescence, and calcification of PI-treated VSCMs.
Conclusions: ZMPSTE24 downregulation is a major contributor in VSMC dysfunctions resulting from *LMNA* mutations or PI treatments that could translate in early atherosclerosis at the clinical level. These novel pathophysiological mechanisms could open new therapeutic perspectives for cardiovascular aging.

Abbreviations: ALP, alkaline phosphatase; ATV, atazanavir; DRV, darunavir; HIV, human immunodeficiency virus; LPV, lopinavir; M/R, maraviroc/raltegravir; PBMCs, peripheral blood mononuclear cells; PIs, protease inhibitors; ROS, reactive oxygen species; RTV or r, ritonavir; Runx2, runt-related transcription factor 2; VSMCs, vascular smooth muscle cells.

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1. Introduction

A-type lamins are intermediate filaments of the nuclear lamina, a filamentous network located below the inner nuclear membrane and required for structural and functional integrity of the nucleus. A-type lamins interact with B-type lamins at the nuclear envelope, as well as to heterochromatin and transcriptional regulators, highlighting their important role in chromatin organization, gene expression, and DNA repair. Alternative splicing of the *LMNA* gene gives rise to two main A-type lamin isoforms, prelamin A and lamin C. Prelamin A undergoes a complex post-translational maturation, initiated by the addition of a farnesyl moiety to the last cysteine residue of the protein. After several steps, removal of the carboxymethylated C-terminal of the protein, including its farnesyl group, by the metalloprotease ZMPSTE24, results in the release of mature non-farnesylated lamin A [1].

Several studies have shown the cellular toxicity of accumulated prelamin A, pointing to the role of the retained farnesyl group, which alters the binding properties of the partially processed prelamin A to several partners [2–4]. Persistent farnesylation of prelamin A has been involved in premature cellular senescence associated with several diseases. This is the case in premature ageing syndromes as Hutchinson–Gilford progeria (HGPS) due to mutations in *LMNA* or *ZMPSTE24* [5–9], but also in *LMNA*-related progeroid and/or lipodystrophic syndromes [10,11].

Mutations in *LMNA* and accumulation of farnesylated prelamin A are cardinal features of typical premature aging diseases, but also of progeroid syndromes and Familial Partial Lipodystrophy of the Dunnigan type (FPLD), all these conditions being associated with early atherosclerosis and cardiovascular events [12–15]. In addition, altered lamin A maturation could be involved in age-related atherosclerotic cardiovascular diseases in the general population. Indeed, prelamin A accumulates in the arterial wall during physiological aging, and colocalizes with degenerating and senescent vascular smooth muscle cells (VSMCs) into atherosclerotic lesions, a defect in part attributed to ZMPSTE24 downregulation [16,17].

Human immunodeficiency virus-1 (HIV)-infected patients display an increased cardiovascular risk, attributed to multiple synergic factors including the therapeutic use of some antiretrovirals belonging to the class of HIV protease inhibitors (PIs), which target the HIV aspartyl protease [18–20]. Interestingly, it has been previously shown that some of these PIs, including lopinavir and atazanavir, and to a lesser extent darunavir, can directly inhibit ZMPSTE24 activity and induce cellular prelamin A accumulation [10,21–23]. In addition, PI-induced farnesylated prelamin A accumulation was associated with cellular dysfunction and senescence in several cellular models [10,24–26]. Until now the potential role of ZMPSTE24 deregulation in *LMNA*-mutated and PI-treated patients, which could participate to their premature vascular aging, has never been investigated.

VSMCs contribute to the initiation of atherosclerosis by transdifferentiating into osteoblast-/chondrocyte-like cells. This process results, amongst other factors, from the activation of osteoblastic transcription factors, like Runx2, and from oxidative and inflammation pathways [27–29]. Calcification of VSMCs, associated with premature senescence [30], could lead to arterial calcification and stiffening [31] as observed in patients with HGPS and *LMNA*-linked progeroid syndromes [12,14]. Coronary artery calcium score, a marker of vascular age and subclinical atherosclerosis [32], is increased in HIV-infected patients [33,34], and long-term antiviral therapy including PIs is associated with vascular calcification [33,35,36].

Therefore, the objective of our study was to explore the potential link between ZMPSTE24 deregulation and the development of VSCM dysfunctions in response to *LMNA* mutations and PI-treatments.

We show, for the first time, that prelamin A accumulation observed in cells from patients harboring FPLD or progeroid syndromes-associated *LMNA* mutations, or treated with long-term PI-based HIV antiretrovirals, was associated with ZMPSTE24 downregulation. We then revealed in cultured VSMCs that overexpression of these *LMNA* mutations or long-term exposure to ritonavir (r)-boosted PIs lopinavir (LPV) or atazanavir (ATV), also induced ZMPSTE24 downregulation together with triggering senescence and associated dysfunctions to different extents, resulting in osteoblastic conversion. These results, showing that ZMPSTE24 downregulation play a major role in the pro-atherosclerotic alterations of VSCMs, suggest novel pathophysiological mechanisms linking lipodystrophy-associated *LMNA* mutations and PI exposure to precocious cardiovascular diseases.

2. Material and methods

2.1. Cell culture and treatment

Human coronary artery vascular smooth muscle cells (or VSMCs) were obtained from middle-aged healthy donors (PromoCell, Heidelberg, Germany) and cultured from passages 2 to 8. The cells were either transfected with void, wild-type (WT) or mutant *LMNA* expression vectors, or exposed for 21 days to clinically relevant concentrations (Cmax) of protease inhibitor (PI) combinations (LPV/r, 15.9/1.4; ATV/r, 7.4/1.3 or DRV/r 11.8/0.8 μmol/L, respectively) (Santa Cruz Biotechnology, CA) [26], or to the solvent (0.1% dimethyl sulfoxide, DMSO). The farnesyl moiety synthesis inhibitor pravastatin (25 μmol/L), the farnesyl transferase inhibitor FTI-277 (20 μmol/L), the antioxydant N-acetyl cysteine (NAC, 1 mmol/L) or anti-calcifying agent etidronate disodium hydrate (4 μmol/L) were added along the PI exposure (pravastatin and etidronate) or for the last 3 days (FTI-277), 4 days (NAC) or 10 days (etidronate), respectively.

2.2. Patients with LMNA mutations or HIV infection

We studied different heterozygous *LMNA* mutations, which substituted arginine to tryptophan at codon 482 (R482W), leading to Familial Partial Lipodystrophy of Dunnigan (FPLD), or aspartate 47 to tyrosine (D47Y) or arginine 133 to lysine (R133L) leading to atypical progeroid syndromes [10,12]. Patients harboring these mutations presented with lipodystrophy, insulin resistance, dyslipidemia, liver steatosis and premature atherosclerosis [10,12,15]. Primary skin fibroblast cultures were established after plastic surgery in four healthy controls (non-obese, non-diabetic) or after punch biopsy in patients with *LMNA* R482W ($n = 6$) [37], D47Y ($n = 1$) [10] or R133L ($n = 1$) [12] mutations. All the subjects gave their written informed consent for these studies.

HIV-infected patients participated to the ANRS157 ROCnRAL study [38]. At inclusion, five patients (median age: 57+/− 9 years) on long-term antiretroviral therapy with suppressed viremia were switched to a regimen combining a CCR5-receptor antagonist (maraviroc) and an integrase inhibitor (raltegravir). Peripheral blood mononuclear cells (PBMCs) were collected at enrollment and after 4 months of maraviroc/raltegravir (M/R) therapy. Proteins were extracted as described [24] and the level of prelamin A and ZMPSTE24 were quantified by Western blotting.

2.3. Transfection assays of LMNA mutants

Flag-tagged wild-type (WT) and D47Y-, R133L- and R482W-mutated prelamin A cDNA were cloned in pSVK3 to yield WT or mutant lamin A expression vectors (GeneCust, Dudelange,