HIV protease inhibitors inhibit FACE1/ZMPSTE24: a mechanism for acquired lipodystrophy in patients on highly active antiretroviral therapy?

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Abstract

HIV-PIs (HIV protease inhibitors) have proved to be of great benefit for the millions of people suffering from AIDS. However, one of the side effects of this component of combined highly active antiretroviral therapy is lipodystrophy, which affects a large number of the patients taking this class of drug. It has been shown that many of these protease inhibitors inhibit the ZMPSTE24 enzyme responsible for removing the farnesylated tail of prelamin A, which is a nuclear lamina component that has been implicated in some of the nuclear laminopathies. Build up of this protein somehow leads to acquired lipodystrophy, possibly through its interaction with a transcription factor called SREBP-1 (sterol-regulatory-element-binding protein-1). The downstream effect of this is altered fatty acid metabolism and sterol synthesis, which may cause lipodystrophy in patients. The build-up of this protein also appears to have morphological consequences on the nucleus and we reveal, by dual-axis electron tomography, a complex nucleoplasmic reticulum that forms after HIV-PI treatment as a result of acute farnesylated prelamin A accumulation. A greater understanding of the molecular mechanisms leading to lipodystrophy will hopefully facilitate the design of improved HIV-PIs that do not cause this debilitating side effect.

Introduction

Nuclear laminopathies are a group of diseases that result from mutations in genes that encode components of the nuclear lamina and associated proteins [1–18]. These rare diseases can be separated into four major classes, namely diseases of striated muscle, peripheral neuropathy, lipodystrophy syndromes and accelerated aging disorders. The LMNA gene generates lamins A and C by differential splicing [19]. Lamin A is initially generated as farnesylated prelamin A, which undergoes a series of post-translational modifications and endoproteolytic cleavages that ultimately result in the removal of the C-terminal farnesylated tail [20–22]. Mutations in the LMNA gene can cause various laminopathies with different degrees of severity based on the position of the mutation in the gene. The most frequent *de novo* mutation in LMNA that has been associated with the rare accelerated aging disorder, HGPS (Hutchinson–Gilford progeria syndrome), activates a cryptic splice site leading to the generation of a truncated farnesylated prelamin A called progerin [14]. The reason for the retention of the farnesylated C-terminus is that the mutation causes the removal of the second cleavage site for the enzyme FACE1 (ZMPSTE24 in mice) [23]. FACE1 is an integral membrane zinc metalloproteinase found in both the nuclear and ER (endoplasmic reticulum) membranes [24,25]. Mouse models of HGPS, in which the ZMPSTE24 enzyme is knocked out (*Zmpste24*$^{-/-}$), show a phenotype that is similar to the clinical symptoms displayed in progeria such as osteoporosis, alopecia and lipodystrophy [26].

FACE 1 is clearly an important enzyme for the correct maturation of lamin A and normal functioning of the nucleus [24]. *Zmpste24*$^{-/-}$ mouse models generate farnesylated prelamin A and some of the consequences of this have been to cause nuclei to adopt a highly dysmorphic shape [27], an alteration of expression in genes that regulate cell cycle progression [28] and a systemic metabolic response involving autophagy induction [29].

Acquired lipodystrophy is a side effect of HAART (highly active antiretroviral therapy)

Within the last 10 years it has been reported that certain drugs seem to inhibit the maturation of lamin A, resulting in the accumulation of farnesylated prelamin A. A well-reported example of this is the use of HIV-PIs (HIV protease inhibitors) that are used to treat some of the 33.2 million people in the world living with AIDS as part of HAART [30–35]. HIV-PIs are designed to inhibit the HIV aspartyl protease

Key words: acquired lipodystrophy, highly active antiretroviral therapy (HAART), HIV protease inhibitor, nuclear envelope, prelamin A, ZMPSTE24.

Abbreviations used: ER, endoplasmic reticulum; HAART, highly active antiretroviral therapy; HGPS, Hutchinson–Gilford progeria syndrome, HIV-PI, HIV protease inhibitor; NPC, nuclear pore complex; NR, nucleoplasmic reticulum; SREBP, sterol-regulatory-element-binding protein.

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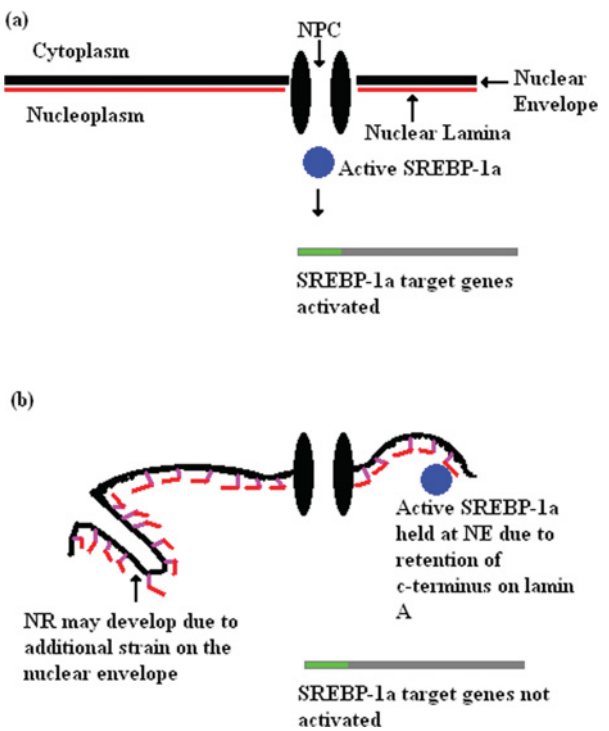
from generating structural proteins for the virus. However, another target of some of the HIV-PIs appears to be the active site of FACE1/ZMPSTE24. This was shown by using an orthologue of ZMPSTE24 (Ste24p from yeast) and treating it with various HIV-PIs, which decreased the enzyme's specific activity [34]. At physiologically relevant concentrations, the HIV-PIs indinavir, nelfinavir, tipranavir, lopinavir and atazanavir cause the accumulation of farnesylated prelamin A around the nuclear envelope [30–35]. Nuclear abnormalities are further confirmed by immunofluorescence microscopy, which shows nuclei with a dysmorphic appearance that are similar to nuclei from HGPS fibroblasts [30]. The abundance of mitochondrial reactive oxygen species also seems to increase in HIV-PI treatment, which may contribute to cells reaching an earlier senescent state and a greater degree of DNA damage, which is an important mechanism leading to premature aging in HGPS [30].

Acquired lipodystrophy, as a result of HAART, was first reported over 10 years ago. It is characterized by the abnormal redistribution of fat tissue around the body, which may lead to both lipohypertrophy and lipoatrophy within 10 months of beginning drug therapy [36] and has 25–75% prevalence among HAART patients [37]. Lipohypertrophy is caused by the accumulation of fat in some parts of the body, such as the belly, upper torso and the back of the neck, leading to the characteristic 'buffalo hump' [38]. Lipoatrophy is due to fat loss in other regions of the body such as the face, buttocks and arms [39]. Psychologically the symptoms can lead to anxiety and depression due to decreased self-esteem, sexual relation problems and general dissatisfaction with body image [40–42]. The iatrogenic consequence of HAART leads many individuals to end up abandoning treatment altogether [43]. The question is how does the accumulation of farnesylated prelamin A, due to inhibition of ZMPSTE24 by certain HIV-PIs, lead to this acquired lipodystrophy syndrome?

Figure 1 | Interaction between active SREBP and prelamin A

(**a**) Schematic of the normal situation within a cell in which during low sterol concentrations the active form of SREBP-1a can move through the NPC and activate target genes. (**b**) When farnesylated prelamin A build up occurs, as a result of acute ZMPSTE24 inhibition, the retained farnesylated tail (pink line) is believed to interact with the membrane resulting in the production of a highly dysmorphic nucleus and the development of a complex NR. Additionally SREBP-1a has been shown to be retained at the nuclear envelope (NE), after farnesylated prelamin A accumulation, preventing activation of target genes involved in adipocyte differentiation and fatty acid metabolism, which may be a possible mechanism for the acquired lipodystrophy in patients on HAART.



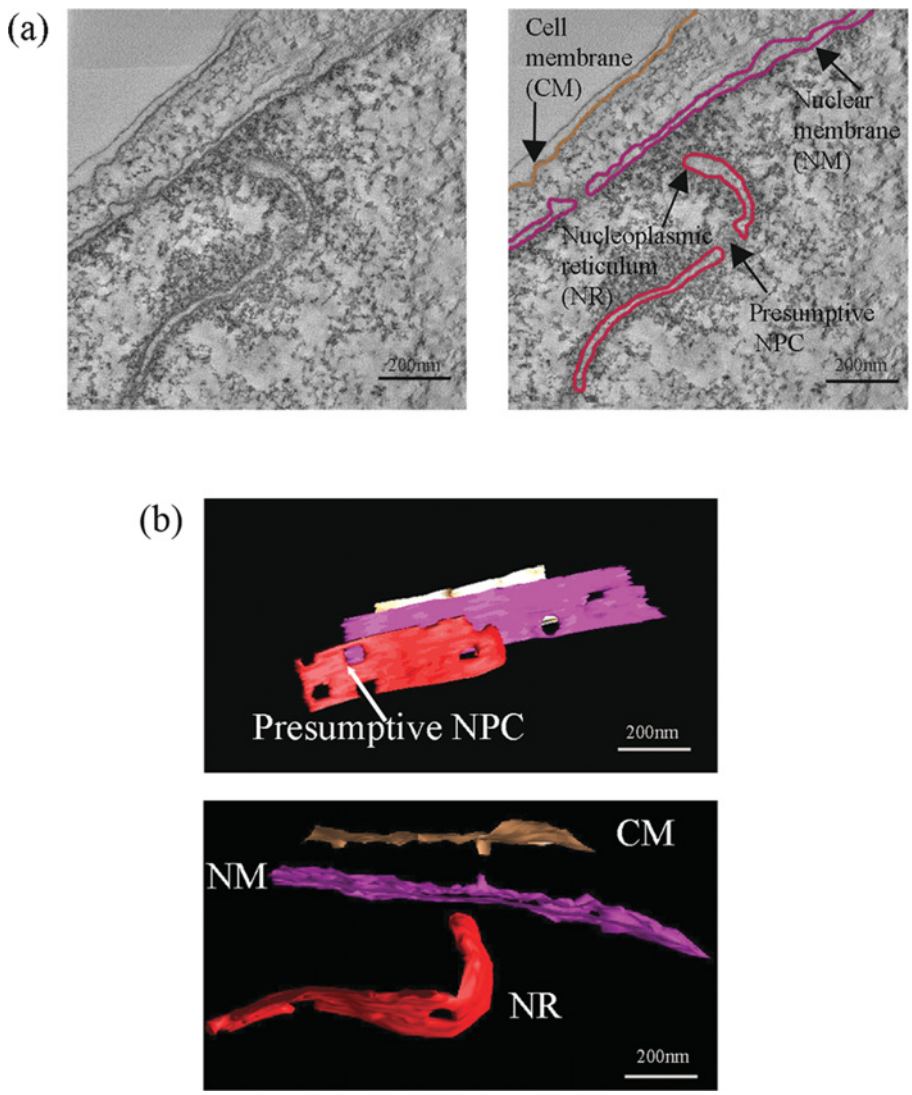
SREBP (sterol-regulatory-element-binding protein) and lipodystrophy

SREBP is a transcription factor that exists in three isoforms (SREBP-1a, SREBP-1c and SREBP-2). SREBP-1a is held in the ER and nuclear membranes, before being activated by periods of low sterol levels in the cell and the mature N-terminus eventually translocates to the nucleus, where it activates genes involved in fatty acid metabolism and adipocyte differentiation [44,45]. Co-immunoprecipitation has shown that the C-terminal fragment, which remains on prelamin A, after inhibiting its cleavage by ZMPSTE24 by using mevinolin, interacts with SREBP-1a [46]. Generation of mature lamin A resulted in no interaction with the SREBP-1a transcription factor [46]. The presence of a farnesylated tail does not seem to make a difference to the interaction between prelamin A and SREBP-1a as immunofluorescence microscopy has shown a nuclear rim staining of the active SREBP-1a in HEK-293 cells (human embryonic kidney cells) transfected with a mutated C661M prelamin A construct (generates unfarnesylated prelamin A) or a mutated L647R prelamin A construct (produces farnesylated prelamin A), rather than in the control situation where it had a more diffuse intranuclear distribution [46]. As HIV-PI treatment also results in the retention of a farnesylated tail on prelamin A, it may offer a possible mechanism for the acquired lipodystrophy experienced in approx. 40% of AIDS sufferers on HAART (Figure 1).

There is currently no treatment for the side effect, although some pharmaceutical companies are developing compounds to tackle this disease; for example, Tesamorelin is a growth hormone, produced by Theratechnologies, which reduced fat levels by 20% [47]. Despite the production of farnesylated prelamin A by certain HIV-PIs, some of the newer generation of HIV-PIs have been found not to cause this unprocessed protein to accumulate. Darunavir has been shown not to inhibit Ste24p and biochemical data revealed no farnesylated prelamin A accumulation of concentrations up to 80 μM

Figure 2 | Dual-axis electron microscopy tomogram of a nucleoplasmic reticulum

(**a**) Dual-axis electron tomography revealed what appear to be invaginations inside the nucleus that appear to contain NPCs with a nucleoplasmic to nucleoplasmic orientation. (**b**) By using modelling software a greater appreciation could be achieved of the structures within the tomogram and this indicates that the NPCs in the NR had similar dimensions to those in the nuclear membrane.



[33]. This obviously offers hope to AIDS sufferers and provides a new approach for the development of these therapeutics.

Conclusion and future perspectives

The research conducted in our laboratory has offered new insights into how these drugs are affecting the morphology of the nucleus by using electron tomography. We have shown that acute administration of an older generation of HIV-PI to mouse embryonic fibroblasts generated a complex NR (nucleoplasmic reticulum) inside the nuclei (Figure 2). The NR is composed of the nuclear envelope containing NPCs (nuclear pore complexes) with a nucleoplasmic to nucleoplasmic orientation. It has been previously reported that the C-terminus of farnesylated prelamin A has been found to interact with the nucleoporin nup53 [48]. In HIV-PI-treated cells it could therefore be envisaged that the inhibition of ZMPSTE24 causes farnesylated prelamin A to build up and the unprocessed protein may then interact with the nuclear membrane as well as other components of the nuclear architecture. This may lead to a highly convoluted and disorganized nuclear membrane. If NPCs are ending up inside the nucleus during interphase, then they cannot be fulfilling their role of transporting molecules across the nuclear envelope, which may add to the pathological symptoms observed inside cells that accumulate farnesylated prelamin A.

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