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PATHOBIOLOGY IN FOCUS

Aging and HIV/AIDS: pathogenetic role of therapeutic side effects

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The intersection of aging and HIV/AIDS is a looming 'epidemic within an epidemic.' This paper reviews how HIV/AIDS and its therapy cause *premature* aging or contribute mechanistically to HIV-associated non-AIDS illnesses (HANA). Survival with HIV/AIDS has markedly improved by therapy combinations containing nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors, and protease inhibitors (PIs) called HAART (*h*ighly *a*ctive *a*nti-ret*r*oviral *t*herapy). Because NRTIs and PIs together prevent or attenuate HIV-1 replication, and prolong life, the population of aging patients with HIV/AIDS increases accordingly. However, illnesses frequently associated with aging in the absence of HIV/AIDS appear to *occur prematurely* in HIV/AIDS patients. Theories that help to explain biological aging include oxidative stress (where mitochondrial oxidative injury exceeds antioxidant defense), chromosome telomere shortening with associated cellular senescence, and accumulation of lamin A precursors (a nuclear envelop protein). Each of these has the potential to be enhanced or caused by HIV/AIDS, antiretroviral therapy, or both. Antiretroviral therapy has been shown to enhance events seen in biological aging. Specifically, antiretroviral NRTIs cause mitochondrial dysfunction, oxidative stress, and mitochondrial DNA defects that resemble features of both HANA and aging. More recent clinical evidence points to telomere shortening caused by NRTI triphosphate-induced inhibition of telomerase, suggesting telomerase reverse transcriptase (TERT) inhibition as being a pathogenetic contributor to premature aging in HIV/AIDS. PIs may also have a role in premature aging in HIV/AIDS as they cause prelamin A accumulation. Overall, toxic side effects of HAART may both resemble and promote events of aging and are worthy of mechanistic studies.
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HIV/AIDS AND AGING, THE SCOPE OF THE PROBLEM

Approximately one million US residents are infected with HIV-1 or have overt AIDS.[1,2] HIV/AIDS survival has been enhanced by nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) in combinations frequently referred to as 'HAART' (*h*ighly *a*ctive *a*ntiret*r*oviral *t*herapy). HAART prevents or attenuates HIV-1 replication and improves survival, making HIV/AIDS a chronic illness.[3] Of note, long-term side effects from antiretroviral agents are poorly understood and may be incompletely recognized as yet, as patients receiving decade long HAART therapy are now growing in number. The population with HIV/AIDS that is surviving into 'senior citizenship' is growing because of those same therapeutic advances, and this argues for the increased prevalence and recognition of important side effects.

Both HIV/AIDS *per se* and its therapy contribute to the phenotype of immune senescence, which is found in aging in the absence of HIV/AIDS.[4–13] A combination of HIV/AIDS and HAART likely exhibits long-term effects on the mitochondrial genome and many of the observed deleterious events result from, are triggered by, or are enhanced by oxidative stress and mitochondrial dysfunction. The interplay of these events is complex and regulation may occur at a variety of cellular levels.

Figure 1 shows the complex interactions that are proven or presumed contributors to aging and HIV/AIDS. A robust interplay occurs between the mechanisms for aging, toxicity of HIV/AIDS therapy, and other events that together serve as a pathogenic foundation for the aging phenotype.[14] This review focuses primarily on side effects of antiretroviral therapy and how those side effects impact development and

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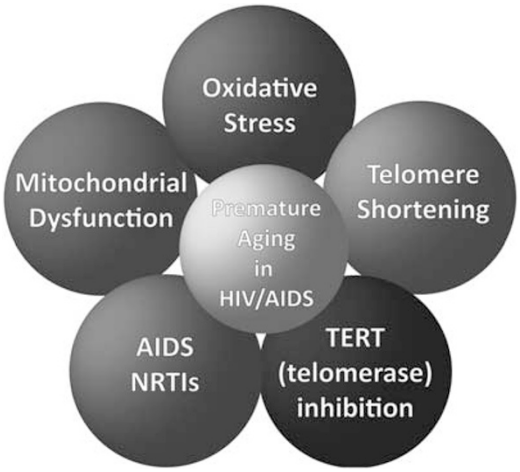

Figure 1 Aging in AIDS results from the interplay of biological events, toxic events, and therapeutic side effects.

prevalence of non-immunologically driven diseases in HIV/AIDS patients. Many of these side effects involve or are tied to mitochondrial dysfunction and oxidative stress. Others have underpinnings in classic theories of aging that are intertwined with metabolic changes in the mitochondria. The interplay contributes to the enhancement of illnesses associated with aging on a 'mitochondrially centered' basis.

Three important theories that explain the aging process are oxidative stress, telomerase inhibition and telomere shortening, and lamin A mutations and accumulations. Each directly, indirectly, or in combination relates to HIV/AIDS and side effects of HAART. For the purpose of this review, aging is defined as 'progressive deterioration of virtually every bodily function over time,' ultimately resulting in death.[15]

OXIDATIVE STRESS

'Oxidative stress' has been used to describe a biological state in which cellular production of reactive oxygen species (ROS) exceeds antioxidant scavenging capacity and results in deleterious events in cells, tissues, and organs. This term has been challenged, because production of ROS can occur in isolated organelles, such as mitochondria, without perturbing the entire cell.[16] Moreover, ROS exhibits both physiological and pathophysiological signaling roles that further complicates interpretation of their effects as deleterious, salutary, or both.[16] In mammalian cells, the major sources of ROS include the mitochondrial electron transport chain (ETC), the NADPH oxidases, xanthine oxidase, and uncoupled nitric oxide synthase enzymes. There is interplay between these, such that excessive production of ROS from one source can activate another.

Oxidative phosphorylation (OXPHOS), the product of the mitochondrial electron transport machinery for ATP production, declines with age.[17,18] Respiration rates and specific activities of ETC complexes I and IV decline as a function of age in both liver and skeletal muscle tissue. This decline in OXPHOS promotes oxidative stress. Reduced transcription of 12S rRNA and cytochrome *c* oxidase mRNA have been demonstrated in the heart and brain of aged mice. Deficiencies in cytochrome *c* oxidase activity in the cardiac and skeletal muscle and brain have been observed in aging along with patterns of altered mtDNA.[19]

Linnane and co-workers[20–22] emphasized that mammals with short lifespans, such as mice, are particularly effective to study mtDNA changes found in aging. Along with features of higher metabolic rates that may contribute to development of mtDNA mutations, inbred strain genetics, and ease of care and husbandry argues for the utility of murine models for studies of aging. Others support a pattern of accumulation of mtDNA deletions in aging animals and human tissues including heart. Conversely, Attardi's group[23] showed that human centenarians have mtDNA mutations near the replication origin that confer longevity, and this may impact mtDNA replication.

Abundant evidence supports the notion that aging is associated with mitochondrial dysfunction, decreased OXPHOS, and oxidative stress.[24–27] At least 10 mtDNA deletions have been observed in tissues (including the myocardium) from a 69-year-old woman with no known mitochondrial disease, suggesting that mtDNA changes in aging are prevalent.[28] These included a common 4977 bp deletion described by Wallace's group[28] in a series of hearts with both ischemic changes and aging. Analogous findings were obtained by others who estimate its prevalence at $\approx 0.1\%$. The presumed random accumulation of mtDNA defects in the aging, failing heart may result in an array of myocytes that produce Linnane's myocardial 'bioenergy mosaic'; however, mtDNA oxidative changes could be more specific.[20] Because of heteroplasmy in mtDNA segregation, the genetic dosage of a defect will have significant impact.

OXIDATIVE STRESS AND HIV/AIDS

Oxidative injury is integral to HIV/AIDS as a potent inducer of viral activation, viral replication, and DNA damage in infected cells.[29–33] Clinically, HIV-1 infection is associated with a decrease in both intracellular and systemic glutathione (GSH).[34,35] This primary decrease in antioxidant defenses is the converse of increased oxidant production, but yields the same functional result.[36,37]

HIV-1 gene products such as HIV-1 transactivator (Tat) cause oxidative stress. In transgenic (TG) mice that express Tat driven by the β-actin promoter, total intracellular GSH declines significantly in liver and erythrocytes.[38] Flores' group[39] showed that the Tat protein decreases SOD2 cellular expression *in vitro*. Because SOD2 is localized in the mitochondria, this lack of SOD2 would increase mitochondrial superoxide levels. Our group showed that HIV-TAT expression that was transgenically targeted to the heart caused severe mitochondrial damage, mtDNA depletion, and heart failure *in vivo*, which supported those previous findings.[40] This depletion of mtDNA will lead to a reduction of proteins in the ETC, which also increases electron leak and superoxide

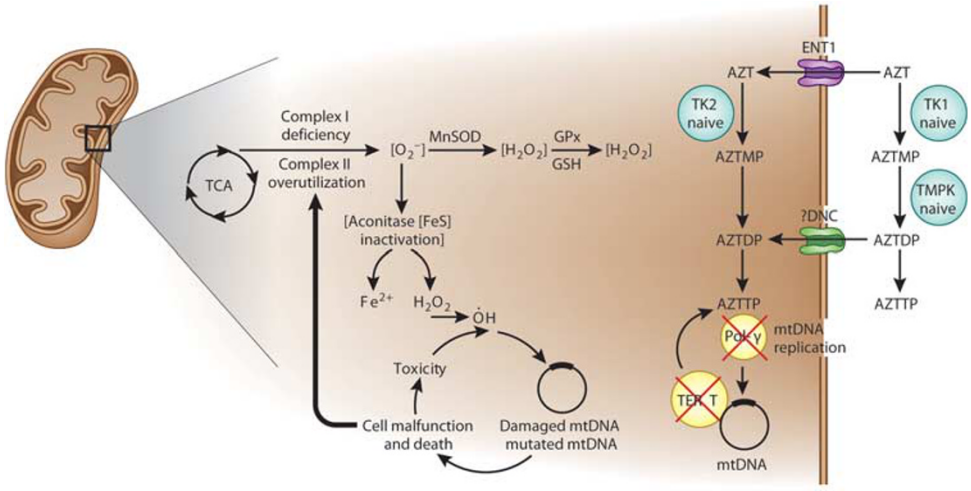

Figure 2 Relationship between mitochondrial dysfunction from HIV/AIDS therapy and mitochondrial DNA replication and mitochondrial telomerase. Both mitochondrial TERT and pol γ are inhibited by AZTTP and other NRTI triphosphates. These interactions may promote the changes of aging, including oxidative stress, mitochondrial dysfunction, loss of TERT protection of mtDNA, and other events. It invokes a new relationship between inhibition of both enzymes and NRTIs in the mitochondria.

production from this source and promotes oxidative stress on that basis.

OXIDATIVE STRESS AND NRTIs

The structural similarity between nucleoside analogs and native nucleosides/nucleotides enables NRTIs to interfere with HIV-1 reverse transcriptase (RT) and inhibit viral replication.[41] Eukaryotic nuclear DNA polymerases that replicate and repair nuclear DNA are less significantly inhibited by NRTI triphosphates than is HIV-1 RT. Among the eukaryotic polymerases, pol γ, the eukaryotic mtDNA replicase is inhibited by NRTI triphosphates at micromolar levels, which is toxicologically relevant.[42,43] NRTI phosphorylation leads to inhibition of mtDNA replication at the level of pol γ, which leads to depletion of mtDNA, oxidative stress, and inhibition of TERT in the mitochondria (Figure 2).[44–46]

Our 'pol γ hypothesis' linked mitochondrial toxicity of NRTIs to inhibition of pol γ, to oxidative stress, and to mtDNA replication defects (Figure 2).[29,30,47] Our group and others showed altered mtDNA replication and decreased energetics are related to toxicity of zidovudine triphosphate (3′-azido-2′,3′-deoxythymidine, AZT) and its pol γ inhibition kinetics.[42,43,48,49] The 'pol γ hypothesis' does not completely explain all toxic manifestations of NRTIs; the exceptions that exist invoke other toxic mechanisms; however, the structure–function relationship for some thymidine analogs as mitochondrial toxins is reasonably established and has held true in clinical trial where mitochondrial toxicity caused cessation.[50]

Early in the HIV/AIDS epidemic, it was recognized that toxicity of NRTIs appeared to target the mitochondria.[51–54] Today mitochondrial side effects are considered relatively common and well established with NRTIs.[55,56] Nonetheless, long-term toxic effects (as may be seen in aging patients treated for decades) are less well studied. These toxic events may relate mechanistically to myocardial infarct, congestive heart failure, liver failure, renal failure, peripheral neuropathy, lactic acidosis, and muscle toxicity in HIV/AIDS (Table 1). Importantly, many of the illnesses are part of the spectrum of diseases seen in aging irrespective of HIV/AIDS.

Not all NRTI compounds contribute directly to mitochondrial toxicity at the level of pol γ. Carbovir triphosphate (2′,3′-dideoxy-2′,3′-didehydroguanosine triphosphate; CBVTP) is an example of a compound that fails to support directly the 'pol γ hypothesis.' Our group has shown that NRTIs contributed to mitochondrial dysfunction in TG mouse models of HIV/AIDS (NL4-3Δ *gag/pol* TG) and that TGs treated with mono-NRTI and HAART and showed oxidative stress was integral to toxic mechanisms.[57–61] The failure of CBVTP to be toxic via this proposed mechanism is mostly likely due to the relatively weak inhibition of pol-γ *in vitro* compared with other NRTI triphosphates.

mtDNA depletion leads to reduction of mitochondrially encoded proteins that have important roles in the ETC for OXPHOS. OXPHOS dysfunction promotes electron leak (uncoupling) and superoxide production as well. Such defects in mtDNA replication and decreased energetics are caused by AZT *in vivo* using various animal models including rats and TG mice harboring genes of HIV, and that are treated with mono-NRTI and HAART combinations.[29,30,54,57,58,60–62]

Pathophysiological mechanisms, as they relate to susceptible mtDNA mutation loci, also have not been elucidated completely, and merit further clinical and experimental study. Using genetically engineered murine models, Suomalainen's group[63] showed that their Polg-Mutator mice had neural (NSC) and hematopoietic progenitor dysfunction from embryogenesis to adulthood because of defective pol γ

Table 1 Aging and NRTI toxicity: clinical features and targets

NRTI	Tissue (mitochondrial) target	Findings related to aging
AZT	Skeletal muscle	Mitochondrial myopathy; depleted mtDNA
	Myocardium	Cardiac failure; mitochondrial cristae dissolution
	Multiple tissues	Lactic acidosis; hyperlactatemia
	Liver	Hepatomegaly, steatosis
	Adipocyte	Lipodystrophy/lipoatrophy/ lipohypertrophy
ddC	Peripheral nerve	Painful peripheral neuropathy
ddI	Peripheral nerve	Painful peripheral neuropathy
d4T	Peripheral nerve	Painful peripheral neuropathy
		Lipodystrophy/lipoatrophy/ lipohypertrophy
FDDA	Myocardium	Dysrhythmia, sudden death
L-FMAU	Skeletal muscle	Mitochondrial myopathy
FIAU	Liver, heart, muscle, pancreas	Acidosis; liver, kidney, heart failure
TDF	Kidney	Mitochondrial tubular disease

activity. Abundance of stem cells was reduced *in vivo*, leading to anemia and lymphopenia, and all are related to ROS-induced dysfunction.[63] As proof of principle, our group showed that cardiomyopathy caused by NRTIs *in vivo* is ameliorated in young mice by overexpression of SOD2 or mitochondrially targeted catalase expression in mice (called mCAT), suggesting that the heart is protected by catalase expression in the mitochondria.[64]

NRTI TOXICITY AND OXIDATIVE STRESS

As mentioned, energy deprivation results from mtDNA depletion that causes defective OXPHOS. This depletion is a cornerstone of theories explaining NRTI toxicity in tissue where OXPHOS is critically important.[57,58] Lactic acidosis and clinical manifestations of energy deprivation occur in patients receiving NRTIs.[65–67] 8-Hydroxydeoxyguanosine (8-OHdG) is an oxidative base product of DNA that reflects oxidative stress and clinical mitochondrial dysfunction; 8-OHdG is present in mtDNA at levels 16-fold higher of those in nuclear DNA.[68,69] 8-OHdG in DNA leads to GC→TA transversions unless the error is repaired.[70] Therefore, 8-OHdG is a potent mutagen that could relate to NRTI toxicity.[71] This is clinically important because trace amounts of 8-OHdG in the mitochondria can markedly reduce DNA pol γ replication fidelity, as suggested by the data from studies *in vitro*.[72]

mtDNA sustains more damage than nuclear DNA in an oxidative event.[73,74] Measurements of 8-OHdG performed experimentally in tissue culture or on isolated mitochondria from animal tissues serve as an index of this form of mtDNA oxidative damage as the proximate target of mitochondrial oxidative stress.[57,58] It has been estimated that the number of oxidative hits to DNA per cell per day is $\approx$100 000 (in rats) and this may relate to mtDNA deletions.[26] Linnane's mtDNA 'bioenergy mosaic' was defined histochemically as a spectrum of mitochondrial activity in tissue (eg, myocardial cytochrome *c* oxidase activity changes with aging).[75] In the nucleus, DNA repair enzymes efficiently remove most of the lesions.[68,69] Such enzyme systems are relatively less effective or absent in the mitochondria to repair mtDNA.[76–78] Oxidative damage to skeletal muscle of mice and rats, and massive conversion of dGuo to 8-OHdG in mice has been attributed to AZT toxicity.[79–81]

Pathophysiological events occur when the threshold of damage impacts organ function, according to the OXPHOS paradigm and the 'pol γ hypothesis.'[82] The importance of mitochondrial oxidative damage is supported by the coexistence of malondialdehyde on (or near) the inner mitochondrial membrane, implying importance of its membrane localization to mitochondrial injury.[83] Malondialdehyde's interaction with mtDNA could lead to crosslinking, errors in transcription, or polymerization, and impact mtDNA biogenesis and replication on that basis.

NRTIs have been used for other viral infections with similar toxic events. The documented anti-HBV activity of fialuridine (1-(2′-deoxy-2′-fluoro-beta-D-arabinofuranosyl)-5-iodouridine; FIAU) was first reported against model hepatitis viruses like duck hepatitis and woodchuck hepatitis virus (WHV).[84–91] Such studies served as preclinical evidence for a clinical trial in patients with chronic active hepatitis B.[92] The resulting tragic clinical experience with FIAU was a significant setback for the therapeutic expectations of the family of antivirals because of FIAU's serious toxicity that included death of some patients.[93] Toxic manifestations of FIAU included profound lactic acidosis, hepatic failure and coma, skeletal and cardiac myopathy, pancreatitis, and peripheral neuropathy.[92] Livers from autopsies and explants from patients who survived and underwent liver transplantation showed marked micro- and macrovesicular steatosis.[92] These clinical data were substantiated by our own studies with FIAU-treated *M. monax* (Eastern woodchuck; WC) infected with WHV in which multiorgan mitochondrial toxicity was found in FIAU-treated WC.[94,95] This led to a retrospective evaluation of trials and a report generated by the Institute of Medicine.[96]

Previous data about D-isomers of FIAU metabolites, FMAU (1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methyluracil) and FAU (1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil), predicted and documented their toxicity on a biochemical basis.[42,97,98] In 2009 a severe toxic mitochondrial myopathy was recognized to be caused by treatment with the L-isomer

known as clevudine (L-FMAU), an enantiomer of FMAU that was considered safe based on studies performed preclinically and early clinical studies.[97,99,100] Toxicity of clevudine caused the abrupt discontinuation of that trial based on findings that included mitochondrial myopathy (resembling that seen previously with AZT) and mtDNA depletion (as seen with AZT and FIAU).[55] Toxicity from this NRTI limited options for millions of patients, and increased morbidity for those already treated. Unfortunately, mechanisms of toxicity in this case remain incompletely understood, although some studies were published that suggested a mitochondrial toxic mechanism that resembles that seen in FIAU.[99,101–104]

Other nucleoside analogs also exhibited significant toxicity and forced cessation of their clinical trials as well. Lodenosine, a purine NRTI (FDDA; 2′-fluoro-2′, 3′-dideoxyadenosine) was considered to be a potentially viable salvage NRTI for HIV/AIDS; however, FDDA mitochondrial toxicity caused cardiac-related death in rats *in vivo* and clinical trials with FDDA were terminated prematurely because of serious adverse events.[105] On the basis of these clinical and preclinical studies, it may be concluded that the 'pol γ hypothesis' is a principle of toxicology in the therapeutic setting of HIV/AIDS and antiretroviral therapy that requires testing.

As recently as 2012, the US FDA placed trials on hold or warned manufacturers of nucleotide analogs for hepatitis C: BMS 986094, IDX184, and GS7977 because of cardiac toxicity events and death, as reported in *The New York Times*.[106] These toxicities appear to be mitochondrially driven, but that theory has not yet been proven conclusively. Other investigators have suggested that the target with this class of compounds may be the mitochondrial RNA polymerase, POLRMT.[107]

TELOMERES AND HIV/AIDS

Telomeres cap the ends of chromosomes and consist of hexameric TTAGGG repeats and the protective 'shelterin' protein complex.[108,109] Telomerase is a ribonucleoprotein consisting of a reverse transcriptase (*TERT*) and its RNA moiety (*TERC*). An 'end replication problem' causes telomeres to shorten during each replication cycle to yield persistent DNA damage and growth arrest (senescence) and limited regenerative capacity of tissues. Its expression causes cellular immortality. Although shortening and/or damage to telomeres is associated with proliferative arrest of cells *in vitro*, it remains unclear how accurately these diseases recapitulate the processes of tissue aging in humans.

All of these enzymes exhibit some evidence of reverse transcription. In the case of HIV-1 RT, this enzyme is capable of catalyzing tRNA-primed DNA synthesis, (−) strand transfer, central polypurine tract-primed (+) DNA synthesis, (+) strand transfer, and ultimately bidirectional DNA synthesis (reviewed in Le Grice[110]). Also HIV-1 RT is capable of continuous and processive nucleotide addition; however, stable complex formation is not involved.[111,112]

Telomerase has been considered the premier eukaryotic RT with putative roles in mitochondrial aging and oxidative stress, aging, and various 'degenerative diseases.'[14,113] Telomerases, including TERT, possess the ability to perform 'repeat addition processivity.' As such, TERT repetitively reverse transcribes a relatively short RNA template. For processive DNA synthesis to occur, the 3′ end of the ssDNA substrate must pair with the telomerase RNA template that creates a DNA–RNA heterodimer. This is reverse transcribed for synthesis of one of the telomeric repeats. DNA synthesis on the same substrate is followed by realignment of the template and repetition of the process.

Mutations reported in TERT have indicated that processivity *per se* is important and that human TERT is more processive than TERT from some other species.[114] Although it is thought to be present in many mammalian tissues, TERT is present primarily in germline cells and is less abundant in mitotically quiescent cells. Experimental evidence suggests that absence of telomerase activity in mice is necessary for telomere length maintenance, but not tumor formation in mice.[115,116]

In vivo experimental systems have been useful to explore mechanisms of aging. Genetically engineered mice that are null for telomerase have been used to examine the role of the enzyme in proliferation and sustainability of neoplastic cells.[117] Others have used knockouts of TERC to refine the roles of stem cells in terminally differentiated, mitotically quiescent cells.[113,118] A relationship between mitochondrial compromise at the level of mtDNA replication defects, TERT inhibition by NRTIs, and resultant telomere dysfunction suggests that a shared mechanism may occur in aging that involves defects in both mitochondrial function and telomere biology. It underscores a relationship between these theories and the observed premature aging that is seen in HIV/AIDS.[14,119]

TELOMERASE AND HIV/AIDS

Telomerase inhibition has been considered a possible mechanism by which antiretroviral treatment in HIV/AIDS causes accelerated aging. At least part of the reasoning behind this hypothesis stems from the fact that NRTIs are known inhibitors of HIV-1 RT, and NRTIs inhibit eukaryotic pol γ (the mtDNA replicase that also has RT activity).[120,121] Early in the HIV/AIDS epidemic, it was discovered that the effect of NRTIs, including AZT, caused progressive telomere shortening in immortalized B- and T-cell lines.[122] More recently, an inhibitory effect of NRTI phosphates on human peripheral blood mononuclear cell TERT indicated that many phosphorylated NRTIs (including lamivudine, abacavir, zidovudine, emitricitabine and particularly the nucleotide analog tenofovir) were inhibitors of TERT.[44] TERT activity is vital to telomerase activity and because of the key role of telomerase in aging theories, it was hypothesized that this inhibition could contribute to the premature aging in HIV/AIDS, and help promote the looming epidemic of premature aging in that population.

Conclusions vary on the importance of TERT activity in HIV/AIDS, and its cellular effects may relate in part to either the cell type, subcellular localization, or both. *In vitro* studies revealed that macrophages (monocyte-derived) when infected with HIV-1 resulted in induction of telomerase activity. These macrophages showed less DNA damage after *in vitro* oxidative stress and may suggest a viral survival strategy that includes making macrophages better suited for survival and thus fostering viral persistence.[123] Evidence also supports decreased TERT activity to be associated with trans-endothelial migration of HIV-1-infected U937 cells. Senescence of brain endothelial cells may worsen many barrier-related functions within the brain and predispose to HIV-1-related inflammatory effects.[124] Aside from cell type, *bona fide* subcellular localization of TERT may be crucial to its function.

MITOCHONDRIA, TERT, AND HIV/AIDS

Mitochondrial localization of TERT has been identified by Santos in Van Houten's group at NIEHS and strengthens the importance of TERT in mitochondrial dysfunction through inhibition of both TERT and pol γ in the mitochondrial matrix (Figure 2).[125] That discovery first suggested that mtDNA repair increased after 6 h in fibroblasts transfected with TERT, however, mtDNA suffered substantial damage and could support apoptosis. Because of its inhibition by AZT triphosphate another toxic mechanism for aging through TERT inhibition in this compartment may contribute pathogenetically as well,[125,126] further underscoring the relationship between TERT inhibition and pol γ inhibition as integral dysfunctional processes (Figure 2).

From a toxicological perspective, the binding of TERT to mtDNA protects against ethidium bromide–induced damage.[127] TERT increases overall ETC activity, which is most pronounced at complex I. Moreover, mitochondrial ROS are increased after genetic ablation of TERT by shRNA.[127] Taken together, this further reinforces the relationship between mitochondrial dysfunction and aging. Other data indicate that in mice that are null for TERT or TERC, there is repression of peroxisome proliferator-activated receptor γ, coactivator 1α and β (PGC-1α and PGC1-β), which suggests mechanistic links exist between metabolic function and aging that warrant further study the setting of HIV/AIDS.[14,119]

Recent evidence associates inhibition of TERT by a number of NRTI triphosphates; other evidence suggests telomere shortening may result from AZT administration to the dam that may have effects on fetal nuclear DNA, suggesting a nuclear telomeric dysfunctional event that may have cytoplasmic and mitochondrial implications.[45,123,128–135] Despite a role for telomere shortening and TERT inhibition, there has not been a direct connection documented between mitochondrially localized TERT and NRTI toxicity.

OTHER ANTIRETROVIRAL SIDE EFFECTS: AGING AND PIs

Premature aging syndromes that clinically appear as accelerated aging in tissues include Werner's and Hutchinson–Gilford Progeria syndromes (HGPS). A-type lamins are nuclear proteins required for the structural and functional integrity of the nucleus. Lamin A is translated as a polypeptide precursor. Mature lamin A is generated after several maturation steps, including C-terminal farnesylation and its removal by proteolytic cleavage.[136] Mutations in the genes responsible for these premature aging diseases result in increased DNA damage, particularly at telomeres, addressed below. Defective maturation of prelamin A is a principal mechanism underlying premature aging as seen in HGPS.[136] Experimental evidence *in vitro* and *in vivo* indicates that retention of the farnesylated residue in partially processed prelamin A confers toxic properties.[137,138] Conversely, a premature aging phenotype in mice is attenuated using inhibitors of farnesylation of prelamin A.[139–143]

Lamin defects also result as a side effect of antiretroviral PIs (used to inhibit the protease of HIV responsible for viral maturation). Two widely used PIs for HIV-1, indinavir and nelfinavir, impede prelamin A maturation *in vitro* in adipocytes. They induce nuclear alterations similar to those observed in *LMNA*-mutated fibroblasts and cause prelamin A accumulation as seen in premature senescence.[144] Thus, antiretroviral therapy combinations likely contribute to aging through a mechanism similar to prelamin A accumulation as well as through oxidative stress.[58,144] These observations argue for previously unrecognized relationship between a PI (used in HAART) and the development of disease-identifying characteristics of senescence in tissues in HIV/AIDS.[144]

Despite their increasing use in HIV/AIDS, side effects of integrase inhibitors do not directly appear to be involved in the aging mechanisms described above.[145] This could relate to the fact that they have only recently been brought into the pharmacopoeia. Evidence exists for a side effect of dyslipidemia, which could be considered a risk factor for increased cardiovascular disease, but are generally acceptable for clinical use.[145,146]

SUMMARY AND PROSPECTS

The aging population with HIV/AIDS has grown because of HAART's therapeutic success and improved patient care, leading to a shift in the number of survivors within the population of HIV/AIDS patients. At present, it remains to be determined whether the root cause of this demographic change is premature aging, 'unanticipated' effects of therapeutic success, or some other factor(s). It is clear that therapeutic side effects and effects of HIV-1 infection together must be considered in the pathogenesis of aging in this population, as antiretroviral therapy is linked directly to the infection.

Mechanisms of premature aging in HIV/AIDS may mimic some of those operative in non-AIDS conditions, and some mechanistic inferences may be made due to telomerase inhibition and other events related to HIV/AIDS therapeutic side effects. Carefully controlled prospective clinical studies will assure that meaningful data are obtained to dissect the clinical nature of each condition that relates HIV/AIDS to aging. Moreover, mechanistic insights will result from basic

studies that explore subcellular events in these intersecting illnesses or complex biological conditions like aging. Taken together, both approaches will illuminate the mechanistic relationship between HIV/AIDS and aging, offer possible ways to therapeutically intervene, and promote human health.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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