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HIV-1 VIRAL PROTEIN R (VPR) AND ITS INTERACTIONS WITH THE HOST CELL

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Abstract

Human immunodeficiency virus type 1 (HIV-1) is engaged in dynamic and antagonistic interactions with host cells. Once infected by HIV-1, host cells initiate various antiviral strategies, such as innate antiviral defense mechanisms, to counteract viral invasion. In its turn, the virus has different strategies to suppress these host responses to infection. The final balance between these interactions determines the outcome of the viral infection and disease progression. Recent findings suggest that HIV-1 viral protein R (Vpr) interacts with some of the host innate antiviral factors, such as heat shock proteins, and plays an active role as a viral pathogenic factor. Cellular heat stress response factors counteract Vpr activities and inhibit HIV replication. However, Vpr overcomes these heat-stress-like responses by preventing heat shock factor-1 (HSF-1) - mediated activation of heat shock proteins. In this review, we will focus on the virus-host interactions involving Vpr. In addition to heat stress response proteins, we will discuss interactions of Vpr with other proteins, such as EF2 and Skp1/GSK3, involved in cellular responses to Vpr, as well as strategies to develop novel antiviral therapies aimed at enhancing anti-Vpr responses of the host cell.

Keywords

HIV-1; Vpr; host cellular responses; viral pathogenesis; heat shock factors; EF2; Skp1/GSK3; host-pathogen interaction

INTRODUCTION

Upon infection by human immunodeficiency virus type 1 (HIV-1), host cells initiate various innate, cellular intrinsic immune responses to counteract the viral invasion. Limited and transient restriction of viral infection is normally achieved. However, HIV ultimately overcomes these antiviral responses resulting in successful viral replication. This is because most of the host innate responses are either too weak or are counteracted by HIV-1. Expression of several HIV-1 regulatory and accessory genes such as *tat, vif*, and *vpu* is known to regulate some of these immune responses to maximize viral replication. For example, Tat was shown to abrogate one of innate immunity mechanisms working at the cellular level, the cell's RNA-silencing defense [1]. Vif inactivates and counteracts the

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function of a cellular deaminase APOBEC3G that affects HIV reverse transcription [2], and Vpu inactivates tetherin (CD317) which inhibits virus release from infected cell [3].

HIV-1 Vpr is a virion-associated accessory protein with an average length of 96 amino acids and a calculated molecular weight of 12.7 kD. Vpr is highly conserved among HIV, simian immunodeficiency viruses (SIV) and other lentiviruses [4,5]. Besides lentiviruses, the Vpr protein sequence shares no strong homology with any other known protein. Vpr displays several distinct activities in host cells. These include cytoplasmic-nuclear shuttling [6], induction of cell cycle G2 arrest [7–9] and cell killing [10]. These three Vpr-specific activities have been demonstrated in a wide variety of eukaryotic cells ranging from yeast to humans, indicating that Vpr most likely affects highly conserved cellular processes.

Host cellular responses to Vpr have not been well described. Understanding the Vpr-specific responses is important because increasing evidence suggests that Vpr plays an important role in the viral life cycle and pathogenesis. In this review, we will describe our current understanding of the role that Vpr plays in viral pathogenesis and the responses of the host cell to Vpr activities.

CONTRIBUTION OF VPR TO HIV-1 BIOLOGY AND VIRAL REPLICATION

Accumulating evidence suggests that Vpr plays an important role in viral replication and pathogenesis. HIV-1 Vpr contributes to viral replication in two different ways. First, it blocks cell proliferation of HIV-infected T-cells by arresting them in G2 phase of the cell cycle [7,9,11], where the viral replication reaches maximum levels [12]. Contribution of Vpr to viral replication in proliferating T-cells, however, is shown to be relatively small *in vitro;* depletion of *vpr* gene from the viral genome typically results in a 2–4 fold reduction of viral replication [12–14]. On the other hand, Vpr is essential for efficient viral replication in non-dividing cells such as macrophages [15,16]. Why the requirement for Vpr differs in these two cell types is not well understood. It is generally believed that the reason why Vpr is required for viral replication in macrophages is because Vpr promotes nuclear import of the viral pre-integration complex (PIC) in non-dividing cells [15,16], thus contributing to the unique feature of lentiviruses that can infect non-dividing cells. However, a recent paper argued against this explanation by showing that infection of growth-arrested T-cells by Vpr(−) HIV-1 was reduced by 2-fold compared to the wild-type virus [17], which was essentially the same level of reduction observed in proliferating cells. In addition, Vpr likely participates in nuclear import of PIC in T cells in a similar manner as it does in macrophages, and nuclear import through the nuclear pore is essential for HIV replication in both cell types [18,19]. Therefore, the differential contribution of Vpr to viral replication in macrophages and T-cells can neither be attributed to the proliferation status nor to the ability of Vpr to promote nuclear import in non-dividing cells. New evidence suggests that the differences in Vpr's contribution to viral replication in different cell types could be due to the presence of different cellular factors that influence Vpr's activity. For example, HSP70 present in T-cells can substitute for the activity of Vpr thus diminishing dependence of viral replication on Vpr in T-cells relative to macrophages where HSP70 is less abundant [20]. Alternatively, there may be a block to HIV replication in macrophages, but not in T cells, that is relieved by Vpr. Indeed, SIV *vpx*, which was derived from African green monkey *vpr* gene by an ancestral recombination event [21], has been shown to overcome an as yet unidentified block to SIV reverse transcription in macrophages [22,23]. Interestingly, to accomplish this goal, Vpx interacts with Vpr binding protein (VprBP)-associated E3 ubiquitin ligase, the same ubiquitin ligase shown previously to be targeted by Vpr to induce G2 arrest in HIV-infected T cells [24]. Therefore, cell cycle stages of infected cells could be a factor in determining the contribution of Vpr to viral replication.

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HOST CELLULAR RESPONSES TO VPR

Since Vpr is a highly conserved viral protein, it presents a good target for host antiviral responses. Indeed, during the acute phase of the viral infection, Vpr is preferentially targeted by the HIV-specific CD8+ T-lymphocytes [25,26]. However, little is known at present about host cell responses to Vpr. Because Vpr acts very similarly in fission yeast (*Schizosaccharomyces pombe*) and mammalian cells, making fission yeast a particularly useful model for studying Vpr activities [39–41]. To study cellular responses to Vpr, we took advantage of the small genome of fission yeast and the flexibility for genetic studies. We first examined the expression levels of cellular RNA transcripts to Vpr using microarray analysis; a genome-wide screening of multicopy Vpr suppressors were then carried out by introducing a fission yeast cDNA library into the *vpr*-expressing fission yeast cells {Elder, 2001; Benko, 2004}. Suppression of a single or all Vpr activities by a protein when it is overproduced was assumed to represent a particular cellular response to counteract a particular or all Vpr activities. This counteracting potential was then compared and confirmed by the results of gene expression microarray analysis. Finally, the inhibitory effect of an identified Vpr suppressor was further verified in the mammalian cells either by expression of Vpr alone or in the context of HIV-1 infection. A set of cellular proteins specifically responding to HIV infection and *vpr* gene expression was identified. These factors include heat shock factor 1 (HSF1)-mediated heat stress-like host responses [13,14,27,28], elongation factor 2 (EF2) [29] and glycogen synthase kinase-3 (GSK3) [30]. Indeed, HSF1 was significantly increased in HIV-infected cells suggesting a possible HSF-mediated host HSP production in response to HIV-1 infection. Comparison of gene expression profiles of HIV-1-infected and heat shock treated cells indicated that HIV-1 infection induced specific changes distinct from a broad activation of stress response pathways characteristic to heat shock [31]. It should be noted that specific cellular activation of HSP27 and HSP70 by HIV-1 infection was initially described by Dr. G. Brenner's laboratory in 1997 [32]. However, that report did not identify the viral protein responsible for HSP elevation. Through the microarray analysis and genome-wide functional screenings of Vpr suppressors, we made the link between Vpr and HSPs [13,14,28] and further showed that HSP activation is mediated through HSFl [27]. Similar findings have been reported by several other laboratories studying Vpr [31,33].

1. Vpr and EF2

A fission yeast translational elongation factor 2 (Ef2), was identified through a genome-wide search for multicopy suppressors of Vpr-induced cell death. Ef2 is an evolutionarily conservative monomeric GTPase involved in protein synthesis and translation elongation in fission yeast and human cells (for review, see [34]). Expression of *EF2* appears to be responsive to *vpr* gene expression in CD4+ T-lymphocytes as moderate increases of EF2 were observed in *vpr*-expressing H9 and CEM-SS cells [29]. Interestingly, expression of fission yeast *Ef2* was also elevated upon *vpr* gene expression [29]. Elevated production of EF2 in both yeast and mammalian cells suggests that Vpr regulates expression of *EF2* through a conserved mechanism. Moreover, overproduction of EF2 blocks Vpr-induced cell death both in fission yeast and human cells, suggesting that this highly conserved activity is to prevent Vpr-induced cell death or apoptosis. The anti-apoptotic property of EF2 has been demonstrated by its ability to suppress caspase 9 and caspase 3-mediated apoptosis induced by HIV-1 Vpr [29]. In addition, EF2 also reduces cytochrome c release induced by three different apoptosis-inducing agents, staurosporine, TNFα and Vpr [29], suggesting that EF2 is a putative anti-apoptotic cellular factor primarily involved in the mitochondria-dependent pathway. Together, the responsive elevation of EF2 to *vpr* gene expression in CD4+ T-lymphocytes and counteraction of Vpr-induced apoptosis by high level of EF2, suggest that EF2 is a cellular factor that may be involved in host innate antiviral response against Vpr.

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2. Vpr and Skp1/GSK3

By using both colony-forming ability and changes of mitochondria1 morphology as endpoints for screening suppressors of Vpr-induced cell death in fission yeast cells, Skpl was identified as a new suppressor for Vpr-induced cell death. Similar to Hspl6, Hsp70, and Ef2, Skpl was initially identified as a possible Vpr suppressor through a genome-wide screen of a fission yeast cDNA library. Over-expression of Skp1 in fission yeast was found to restore cell survival to 70% in the presence of *vpr* expression[35]. Furthermore, expression of Skp1 partially restored morphology and normal distribution of mitochondria in the *vpr*-expressing yeast cells [35], suggests that Skp1 has suppressive activity not only against Vpr-induced cell death, but also against mitochondrial abnormalities induced by Vpr.

Based on protein sequence alignment, fission yeast Skpl is most likely the homologue of mammalian glycogen synthase kinase-3 (GSK3). Roughly 60–64% of the protein sequences are identical, and 75–78% of the amino acids are positively correlated over the entire proteins. GSK3 is a member of a highly conserved family of protein serine/threonine kinases that are involved in the regulation of a diverse array of cellular functions, including protein synthesis, cell proliferation and differentiation, microtubule assembly/disassembly, and apoptosis (for reviews, see [36,37]). However, the specific role of Skpl/GSK3 in apoptosis is unclear and complex. In fact, GSK3 appears to play both pro- and anti-apoptotic regulatory roles. GSK3 promotes mitochondria-dependent apoptosis, but it inhibits apoptosis induced by the death receptor-mediated signaling pathway [38]. Since GSK3 has two isoforms (α and β), it is currently unclear whether both isoforms have the same regulatory activities on apoptosis or each of the isoforms has its unique regulatory role in apoptosis. Further characterization of fission yeast Skpl, its mammalian counterparts, and their role in Vpr-induced apoptosis will certainly shed light on the important regulatory mechanisms of Skpl/GSK3 in apoptosis.

3. HSF1-mediated host cellular responses against Vpr

1) Vpr and small heat shock proteins (sHsp)—Using Vpr-induced cell cycle G2 arrest and cell death as a target, several fission yeast heat shock genes, including Hsp16 and Hsp70, were identified as multicopy suppressors of Vpr activities [13]. Further studies showed that Hsp16 specifically inhibits the Vpr activities [42,43]. These suppressing effects seem to be a conserved phenomenon as Hsp16 showed similar suppressive activities toward Vpr in yeast and mammalian cells [13]. In addition, Hsp16 directly associates with Vpr [13]. Expression of HIV-1 Vpr protein in budding yeast has been also reported to cause growth arrest [44,45]. Investigation of the Vpr effects in this yeast showed disruption of the actin cytoskeleton. Expression of hsp42, another sHsp, from a high-copy-number plasmid reversed this effect [33].

Comparison of protein sequences of fission yeast Hsp16 and human Hsp27 suggests that Hsp27 might be a functional paralogue of Hsp16 because they both share the same motif of the Hsp20/α crystalline family [46]. In HIV-infected cells, up-regulated expression of *hsp27* mRNA was observed as early as 3–8 hr following viral infection [47,48]. Notably, *hsp27* mRNA transcripts were down-regulated by 24 hr, concomitant with the first appearance of full-length genomic HIV-1 mRNA [32]. The HIV-induced up-regulation of Hsp27 shortly after HIV infection is due to Vpr activity [28,32,47]. Indeed, expression of *vpr* either alone or in the context of HIV infection elicits a transient increase of the Hsp27 protein level, whereas Vpr-defective HIV infection induced only a small increase in Hsp27 [28,32].

Responsive elevation of fission yeast Hsp16 and its human paralogue Hsp27 [28] suggests that the cellular heat stress-like responses might be antagonistic to Vpr. However, the

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suppressive effects of yeast Hsp16 and human Hsp27 on Vpr are not identical. Overproduction of Hsp16 completely eliminated all of the Vpr activities including the positive role of Vpr in supporting viral replication in macrophages [27]. Under the same conditions, however, Hsp27 had no clear suppressing effect against Vpr in macrophages [27]. At the functional level, Hsp27 reduces Vpr-specific cell cycle arrest and apoptosis [28], but has only a marginal effect on Vpr-mediated nuclear import [14,49]. One possible difference between these two sHsp's is that Hsp16 associates directly with Vpr [13] whereas no clear Hsp27-Vpr interaction was detected either *in vitro* or *in vivo* [28]. Since Vpr is particularly important for HIV-1 infection of macrophages [6,16,50], the inability of Hsp27 to block nuclear import of Vpr could potentially explain why it has no effect on HIV-1 infection in macrophages. Together, these data illustrate a dynamic and antagonistic interaction between HIV-1 Vpr and a host cellular protein Hsp27, suggesting that Hsp27 may contribute to cellular intrinsic immunity against HIV-1 infection.

2) Vpr and Hsp70—Shortly after HIV-1 infection, the level of Hsp70 significantly increases in different cell types, including MAGI, PBMCs and macrophages, providing a certain degree of protection against HIV-1 infection [14,49]. This protective effect is most evident in macrophages subjected to heat shock, likely because Hsp70 levels induced by the heat shock are significantly higher than after HIV-1 infection. It is possible that other heat-shock proteins, such as Hsp27, also contribute to the observed protective effect [49]. Heat shock and overexpression of Hsp70 reduce HIV-1 replication in macrophages and H9 cells, whereas suppression of Hsp70 by RNAi increases the Vpr-dependent viral replication, suggesting a specific Hsp70-Vpr interaction [14,49]. Indeed, Vpr specifically binds to Hsp70 in HIV-1–infected cells which may explain the neutralizing effect of Hsp70 on Vpr activity. Another possibility is that Hsp70 may counteract the nuclear import activity of Vpr thus reducing HIV-1 replication [14,49]. These findings extend the observation that overexpression of Hsp70 reverses the G2 arrest induced by HIV infection in HeLa cells. These inhibitory effects of Hsp70 on Vpr-dependent G2 arrest, apoptosis, nuclear import and HIV-1 infection are consistent with Hsp70 functioning as an innate anti-HIV factor.

Surprisingly, Hsp70 also stimulates nuclear import of PIC and viral replication in macrophages of Vpr-deficient HIV-1 [49]. This observation prompts the idea that Hsp70 may be able to replace Vpr of HIV-1 during nuclear import of the viral pre-integration complex [20]. In particular, Vpr, similar to the effect of Hsp70, enhances karyophilic properties of weak NLSs present in the matrix protein, thus stimulating nuclear import of the HIV-1 PIC [51]. This effect is consistent with the capacity of Hsp70 to stimulate nuclear import of weak karyophiles [52]. Interestingly, in H9 cell lines, where Vpr has small effect on HIV replication, neither overexpression nor suppression of Hsp70 showed significant effect on viral replication, suggesting that suppressive activities of Hsp70 preferentially target Vpr [14]. It should be noted that H9 cells are proliferating cells, whereas Vpr-dependent nuclear translocation is especially critical in nonproliferating macrophages [53]. Therefore, while active nuclear import seems to be important for HIV-1 infection of both proliferating and nonproliferating cells [54], the role of Vpr (and Hsp70) in HIV-1 nuclear transport in dividing cells remains elusive [14].

3) Vpr and HSF1—HSF1 and HSF2 are upstream regulators of HSP-mediated stress responses (for review, see [55]). Hsf1 is the main regulator responsible for Hsp16 elevation in fission yeast cells. Overexpression of *hsf1* completely inhibited Vpr-induced cell cycle G2 arrest and additional heat treatment of *hsf1*-expressing cells did not significantly enhance the suppressive effect of Hsf1 on Vpr [27]. These findings indicate that Hsf1-dependent Hsp16-mediated suppression of Vpr is the key component of the cellular heat shock response involved in anti-Vpr activity.

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The stimulating effect of Vpr on Hsp27 expression might also involve HSF1. Although the mechanistic details of Vpr-dependent induction and restriction of HSF1 expression remain to be further delineated, the finding that *vpr* gene expression elicits HSF1, but not HSF2, activation [28] suggests that Vpr triggers a cellular stress response that may specifically involve HSF1-dependent cellular events. Indeed, studies have shown that HSF1 and HSF2 are characteristically different in their regulation of stress responses [55]. HSF1 is a rapid responder to heat-related stresses, whereas HSF2 is an active responder to hemin treatment but refractory to heat stress [55–57]. Therefore, it is reasonable to assume that Vpr may activate a cellular stress response that is similar to heat shock response. Because HSF1 modulates heat shock protein by transcriptional regulation [55–57], it is likely that the HSP27 response to Vpr is regulated at the transcriptional level. This notion is certainly supported by the studies in fission yeast showing that activation of Hsp16 occurs at the transcriptional level [27].

COUNTERACTION OF HOST IMMUNE RESPONSES BY VPR

All regulatory and accessory HIV-1 proteins are targeted by HIV-1-specific CD8-positive cytotoxic T-lymphocytes (CTLs) [58]. However, Vpr is preferentially targeted by the CTL response in comparison to other viral proteins, at least during the acute phase of infection [25,26], suggesting an important role for Vpr during the early phase of infection. Vpr suppresses antigen-specific CD8-mediated CTL and Th1 immune responses [59]. Consistent with the idea that Vpr suppresses the immune response, rhesus macaques infected with HIV-2 lacking the *vpr* gene had increased antibody titers compared to monkeys infected with the wild-type virus [60]. Although the molecular mechanisms underlying the suppression of CTL and antibody production by Vpr are presently unknown, one possibility is that Vpr inhibits T-helper activity by suppressing T-cell proliferation and inducing cell cycle G2/M arrest [61].

Evidence also suggests that Vpr may suppress host inflammatory responses, which present another level of the host immune responses to viral infections (for review, see [62]). Vpr inhibits host inflammatory responses by down-regulating pro-inflammatory cytokines (TNFα and IL-12) and chemokines (RANTES, MIP-1α and MIP-1β) in a manner similar to glucocorticoids [63,64]. Vpr additionally suppresses host inflammatory response by inhibiting NF-κB activity through the induction of IκB [64].

Vpr also appears to suppress cellular innate immune responses (intrinsic immunity) involving some of the cellular chaperone proteins. An example of this activity is Vpr-mediated counteraction of heat-stress-like responses by down-regulation of heat shock proteins. For example, even though *vpr* gene expression triggers Hsp16 elevation, Vpr appears to prevent elevation of Hsp16 induced by heat treatment [27], suggesting a counteracting effect of Vpr possibly through transcriptional regulation of *hsp16*. This is supported by the observations that induction of Hsp16 by heat treatment failed to counteract Vpr-induced cell death [27]. However, overexpression of *hsp16* under the control of an exogenous *nmt1* promoter completely suppressed Vpr-induced cell death under the same heat shock conditions [13,27]. This suggests that the observed transcriptional down-regulation of *hsp16* by Vpr is responsible for the ineffective suppression by heat shock of *vpr* induced cell death. Although the molecular mechanism underlying the transcriptional suppression of *hsp16* by Vpr is unclear at the moment, the findings that Hsf activates Hsp's through binding to the Hsp promoters [65] and overexpression of *hsf1* or *hsp16* through an exogenous *adh* or *nmt1* promoter alleviates the Vpr suppressive effect [13,27] support the idea that Vpr may affect expression of *hsp16* through competition with Hsf for control of *hsp16* promoter. One possible scenario is that Vpr may bind Hsf1 preventing it from activating transcription of *hsp16*. Alternatively, since Vpr is a weak transcriptional activator

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through binding to the transcriptional factor Sp1 [66], it is also possible that Vpr may compete with Hsf1 by binding to the Sp1 region of the *hsp16* promoter. Interestingly, only the wild type Vpr was able to inhibit Hsp16 at early hours (23 hrs) after induction, while a single amino acid substitution from phenylalanine to isoleucine at position 34 of Vpr attenuated its ability to suppress the increase of Hsp16 after acute heat shock [27]. In fact, an even higher level of Hsp16 was observed in that case. This is presumably due to the inability of Vpr-F34I to compete with Hsf for binding to Hsp16 promoter. Since amino acid substitution at residue 34 of Vpr diminishes the ability of Vpr to induce cell death but retains induction of G2 arrest [42,67], a plausible possibility is that suppression of Hsp16 and induction of cell death by Vpr share common pathways.

Similar to effects in fission yeast, Vpr also appears to counteract the antiviral response of Hsp27, possibly through similar transcriptional regulation. This premise is supported by the finding that responsive natural elevation of Hsp27 is not sufficient to restrict the Vpr activities, whereas overexpression of Hsp27 under the control of an exogenous and constitutive promoter overrides the suppressive effect of Vpr [28]. Moreover, transcriptional inhibition of Hsp27 by HIV infection has been demonstrated [32,47], providing additional support to the idea that Vpr antagonizes Hsp27 at the transcriptional level.

Therefore, there appears to be at least two levels of host responses to *vpr* gene expression: one is the cellular immune response mediated by CD8+ CTLs; and the other is an innate immune response involving some of the cellular chaperone proteins. Vpr counteracts both these host responses: it prevents T-cell proliferation, suppresses host inflammatory responses including production of cytokines and chemokines, and downregulates production of heat shock proteins, which have specific suppressive activities against Vpr. These specific host responses to Vpr and the counteracting effect by Vpr strongly suggest a very dynamic interaction between *vpr* gene expression and the host. Future studies should reveal to what extent these interactions contribute to the success of viral infection and will determine the best way to exploit the specific host responses to optimize strategies aimed at suppressing Vpr.

DEVELOPMENT OF POTENTIAL VPR-TARGET ANTI-HIV DRUG THERAPY

Vpr plays a pivotal role in viral pathogenesis since Vpr activities are linked to promotion of viral infection in non-dividing macrophages and monocytes, prevention of T-cell clonal expansion, and depletion of CD4 T-lymphocytes. Therefore, strategies to inhibit these adverse Vpr effects could potentially alleviate the impact of the virus and benefit infected patients. Specifically, some of the current anti-HIV drugs are very sensitive to the cell cycle stage of the HIV-infected cells [68–72]. For example, HIV-infected cells arrested in G2 phase of the cell cycle show a significantly decreased sensitivity to the nucleoside reverse transcriptase (RT) inhibitors AZT and d4T, and to a nonnucleoside RT inhibitor nevirapine [68]. Moreover, when HIV-infected cells were shifted out of the G2 phase by cytostatic drugs, such as hydroxyurea or rapamycin, a significant enhancement of drug efficacy was observed [71,72]. Therefore, finding cellular factors that are capable of restricting Vpr activities could provide an opportunity for possible alternative anti-HIV therapy. Since overproduction of HSPs could reverse Vpr-induced G2 arrest in HIV-1 infected cells, at least temporarily [13,28,49], HSPs activated in response to Vpr (HSP27, HSP70 or Hspl6) can potentially be used as an adjunct agent for boosting the anti-HIV activity of certain drugs, such as AZT, d4T or nevirapine. The yeast Hspl6 is the most valuable candidate in this regard. Our unpublished data show that HSP16 synergizes with AZT in PBMCs. In addition, HSP16 shows a very potent anti-Vpr activity and is non-toxic to human cells. Moreover, results of our early experiments show that it can be efficiently taken up by cells and its suppressive effect against Vpr lasts for up to several days [13,27]. The EF2 is another

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potential candidate, which was isolated from both fission yeast and mammalian cells that specifically suppresses Vpr-induced apoptosis [29]. In addition, a number of hexameric peptides with a di-tryptophan motif were found by genetic selection in budding yeast to suppress Vpr-induced G2 arrest and apoptosis in T-cells [73]. These Vpr-specific inhibitors provide leads for the development of anti-HIV therapies with the potential to benefit HIV-infected patients in the future.

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