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News and Commentary

HIV: no PUMA no death?

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The presence of syncytia-inducing (SI) virus, and the formation of multinucleated cells (syncytia) is supposed to play a major role in the progression of infection with the human immunodeficiency virus (HIV). SI viruses, which use the chemokine receptor CXCR4 to infect cells expressing the CD4 molecule (the main receptor for HIV, present on T-helper lymphocytes, monocytes and macrophages), are found more frequently in patients with an advanced stage of the disease than the non-syncytia-inducing (NSI) type, which are usually isolated in the asymptomatic period of the infection. SI HIV isolates have a greater capacity to kill infected cells in culture than do NSI strains, and the switch from NSI to SI is considered a marker of disease progression and an unfavourable prognostic sign. Formation of syncytia causes cell death with features of either apoptosis or necrosis. As far as lymphocytes or monocytes are concerned, syncytia are formed by the fusion of infected with uninfected CD4+ cells: in culture, their formation is one of the first signs of HIV infection of peripheral blood mononuclear cells (PBMC), appearing 2–3 days after adding viral particles or infected biological material (such as cells or plasma). In T-cell lines such as MT-2, the formation of syncytia can occur as early as 2 h after the infection.[1] Cell fusion is temperature-dependent and does not require DNA, RNA or protein synthesis. It involves carbohydrates and lipids present on the cell membrane and depends on CD4 and the HIV gp120 and gp41 envelope proteins.[2]

As far as the progressive loss of CD4+ cells occurring in HIV+ patients is concerned, the real role of syncytia formation is still a matter of debate. Indeed, evidence for the presence of multinucleated cells *in vivo* is lacking except in the brain.[3] HIV infects and induces syncytium formation in microglia cells from the central nervous system (CNS). Macrophages/microglia cells are the main reservoir for HIV in the CNS, and multinucleated giant cells, the result of fusion of HIV-infected microglia and brain macrophages, are the neuropathologic hallmark of HIV dementia.

The formation of a syncytium leads to a cytopathic effect with a typical balloon degeneration of the cells, which is caused by a variety of mechanisms. Among these, those related to triggering of apoptotic pathways play a major role. The growing understanding of p53 function helped to establish its fundamental role in the regulation of apoptosis in different contexts. Many viruses encode proteins that can specifically escape p53-mediated apoptotic programmes (i.e. adenovirus, human papilloma viruses, SV40, etc.).[4] In contrast, HIV is able to hijack the apoptotic machinery of the infected cell to induce its demise, possibly to facilitate virus spreading and counteract either the innate or acquired immune response of the host.[5] Recent reports demonstrated that p53 and its target gene Bax are involved in the induction of apoptosis triggered by HIV in infected primary lymphocytes.[6,7] The role of p53 in HIV-induced cell death has now been more firmly validated by a recent report by Perfettini *et al.*[8] in the Journal of Experimental Medicine. The same group had previously demonstrated the existence of a sequence of events during syncytia formation:[9] (i) activation of mammalian target of rapamycin (mTOR); (ii) mTOR-dependent phosphorylation of p53 at serine 15 and the induction of its target gene Bax; (iii) activation of a mitochondrial death pathway. This process also results in deregulated mitosis through the induction of cyclin B1. However, the exact mechanisms regulating these events are still unclear. To dissect the pathways involved in syncytia formation, the authors analyse the transcriptional activities of several transcription factors upon cell fusion. The activities of p53, NF-κB and AP-1 are strongly enhanced by cell fusion, thus suggesting that these factors are functionally involved in this process. Strikingly, AP-1 activity was abrogated upon transfection of either p53 dominant-negative or NF-κB dominant-negative mutants. Thus, p53 and NF-κB are upstream regulators of AP-1. NF-κB is activated through the phosphorylation and ubiquitin-mediated degradation of its inhibitor IκB.[10] IκB phosphorylation is present in prekaryogamic or karyogamic syncytia, thus indicating that NF-κB is active at both stages of syncytia formation. In contrast, phosphorylation of p53 at Ser 15 and 46, which are markers of its activation, is induced only in early and late karyogamic syncytia, respectively. Inhibition of NF-κB blocks the transcriptional activation of p53 and the emergence of karyogamic syncytia (Figure 1). The induction of cyclin B1 is also inhibited in this context. Based on this evidence, NF-κB regulates the cyclin B1-mediated entry into karyogamy that occurs upstream p53 activation.

These experiments are validated by the observation that IκB phosphorylation is detected in lymph nodes from HIV carriers, mainly among mitotic cells. The involvement of NF-κB in syncytia formation is intriguing, but several questions remain unanswered. Firstly, it is unclear if NF-κB has any proapoptotic activity *per se* and how it regulates p53 phosphorylation and activation. One previous report by the group of Karen Vousden has proposed that NF-κB is induced upon p53 activation and is required for apoptosis induced by p53 in tumour cell lines.[11] Secondly, which are the NF-κB target genes involved in syncytia formation? It is unclear if the canonical NF-κB-dependent antiapoptotic programme is induced upon syncytia induction. Finally, it would also be

important to study the DNA-binding activity of NF-κB by electrophoretic mobility shift assay and chromatin immunoprecipitation to determine which NF-κB dimers bind to DNA upon syncytia formation.

The authors then move on to consider the role of p53 in karyogamic syncytia. First, they find that syncytia formation induced by Env-CD4 interaction in Hela cells has a dramatic effect on gene expression. In all, 82 genes were found to be significantly up- or downregulated. Pifithrin-alpha, a known chemical inhibitor of p53,[12] prevents 85% of these changes in gene expression. Thus, p53 is an essential modulator of the transcriptional programme induced by cell fusion. However, it remains to be established if transcription is essential for syncytia formation. Moreover, it is unknown at present if p53 affects transcription of the deregulated genes directly or indirectly. It is unlikely that p53 would act directly in all cases. The striking effect of the p53 inhibitor pifithrin-alpha on this type of the cell death could have future therapeutic implications. However, inactivation of p53 results in a dramatic increase in the oncogenic potential of Kaposi's sarcoma-associated herpesvirus cyclin, suggesting that inactivation of p53 might result in increase Kaposi incidence *in vivo*.[13]

In order to assess which p53 target genes are affected upon cell fusion, Perfettini *et al*. employ a p53 microarray that includes most known p53 target genes. They find that two main proapoptotic target genes are induced: PUMA and BAX (Figure 1). PUMA is a Bcl-2 family member recently added to the growing list of BH3-only proteins,[14] and is a potent proapoptotic factor that is rapidly induced by ionizing radiation in a p53-dependent fashion.[14] Two recent reports demonstrated that the inactivation of PUMA in the mouse results in no overt effect on development.[15,16] By contrast, PUMA$-/-$ thymocytes and mouse embryo fibroblasts are resistant to ionizing radiation-induced cell death, thus implicating PUMA as the main p53 target for apoptosis.[15,16] Perfettini *et al*. demonstrate that PUMA is induced upon syncytia formation in cell lines and that PUMA is expressed at high levels in lymphocytes infected with HIV *in vitro*. PUMA is also overexpressed in lymph nodes from HIV patients and its expression levels positively correlate with HIV titres. Moreover, cells from patients treated with HAART show reduced levels of PUMA. From a clinical point of view, this observation is of great importance as it indicates that variations in PUMA expression can become a useful marker of the progression of infection, and thus could be used to monitor the efficacy of antiretroviral treatments. PUMA induction correlates with Bax and Bak conformational change and activation. Importantly, syncytial apoptosis is strongly reduced when PUMA, Bax or Bak are downregulated, as demonstrated by using antisense oligonucleotides or RNA interference. Interestingly, another BH3-only family member, Bim, has also been implicated in HIV-induced apoptosis.[17]

It remains unclear whether mTOR upregulation has effects other than on p53 activation, as is also the role of mTOR in

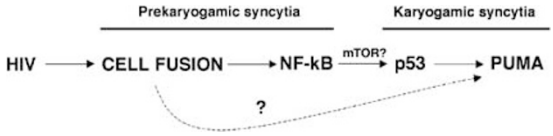

Figure 1 Cell death in syncytia. Syncytia are formed by the fusion of infected with uninfected CD4 + cells. Cell fusion triggers a sequence of events which ultimately lead to cell demise: 1) NF-κB is activated and promotes activation of mTOR; 2) p53 is phosporylated at Ser 15 and 46 in a mTOR-dependent and independent fashion; 3) PUMA is induced and mediates apoptosis. PUMA can also be induced in a p-53 independent manner.

apoptosis.[18] mTOR is part of the fundamental PI-3K/AKT pathway, which is normally activated by growth factors and mitogenic stimuli.[19] Upon activation by AKT, mTOR regulates protein synthesis through the modulation of S6 kinase and the subsequent phosphorylation of the ribosomal subunit S6, and through the activation of the initiation factor of translation eIF4E.[19] Further investigation is needed to assess whether the syncytia-induced activation of mTOR is PI3-K/AKT-dependent and which are the targets of mTOR in this process.

The identification of major players in syncytia-induced cell death is a significant step in understanding the molecular mechanisms of HIV cytotoxicity. However, some outstanding questions require an urgent answer. First, to what extent does syncytia formation occur *in vivo*, and are they a cause of death of infected lymphocytes, as occurs in the CNS? Second, do viral pro-apoptotic proteins such as Tat, vpr and pr play any role in syncytia-induced apoptosis *in vivo*; for example, does the secreted form of Tat play any role in bystander cell death? Third, how can PUMA be used as a marker to monitor the progression of the infection and the efficacy of its treatment?

This paper uncovers an emerging role for p53 and p53 target genes in HIV-induced apoptosis. Future studies will be necessary to gain a more complete understanding of the role of p53 in HIV-induced apoptosis *in vivo*.

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