



VIEWPOINTS

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WIP1 deficiency inhibits HTLV-1 Tax oncogenesis: novel therapeutic prospects for treatment of ATL?

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Abstract

Attenuation of p53 activity appears to be a major step in Human T-lymphotropic virus type 1 (HTLV-1) Tax transformation. However, p53 genomic mutations are late and rather infrequent events in HTLV-1 induced Adult T cell leukemia (ATL). The paper by Zane *et al.* shows that a mediator of p53 activity, Wild-type p53-induced phosphatase 1 (Wip1), contributes to Tax-induced oncogenesis in a mouse model. Wip1 may therefore be a novel target for therapeutic approaches.

Keywords: HTLV-1, Tax, HBZ, p53, Wip1, PPM1D, MDM2, DNA damage response, Genomic stress, ATM, Chk2

Background

Human T-lymphotropic virus type 1 (HTLV-1) is a δ -retrovirus that infects about 25 million people worldwide [1,2]. HTLV-1 causes Adult T-cell leukemia-lymphoma (ATLL) and a neurodegenerative disease called HTLV-induced myelopathy - tropical spastic paraparesis (HAM/TSP). Only a minority of infected subjects (~4%) will develop either ATL or HAM/TSP usually after several decades of latency. Although the mechanisms are still incompletely understood, experimental evidence shows that two factors, Tax and HBZ, are major players in viral replication and oncogenesis [3-5]. The HBZ gene is transcribed from the proviral complementary strand initiating from the 3'-LTR promoter. HBZ activates JunD transcription and TGF β signaling but inhibits the canonical NF- κ B pathway and 5'-LTR-directed transcription [4]. Unexpectedly, HBZ RNA was reported as sufficient to promote T cell proliferation. The other HTLV-1 oncogene, Tax, is a transcriptional activator of viral expression that modulates major intracellular signaling pathways (NF- κ B, CREB and AP1) [1]. Tax immortalizes cells *in vitro* and induces tumors in transgenic mouse models. Tax stimulates S phase progression by accelerating early firing of late replication origins [6]. This process thus permits faster cell proliferation but also engenders DNA strand breaks due to unscheduled replication timing. This mechanism creates an oncogenic stress that triggers the DNA damage

response (DDR) [7]. Concomitantly, Tax also attenuates the DDR by interacting with different components of the p53 pathway such as Chk2 [8] (Figure 1). The p53 tumor suppressor controls cell cycle arrest allowing DNA repair and, if damage cannot be repaired, induces apoptosis or senescence. p53 is thus a major player that directs cell fate upon infection by HTLV-1, as observed for a broad variety of other viruses. The mechanisms by which Tax attenuates p53 activity are still debated, but clearly depend on the cell types and the experimental settings. Nonetheless, it is evident that the inactivation of p53 by gene mutation is less frequent in ATL compared to other human neoplasms (17-42% versus 60%) [9]. Together, these observations enlighten the importance of Tax-mediated inactivation of p53 in ATL.

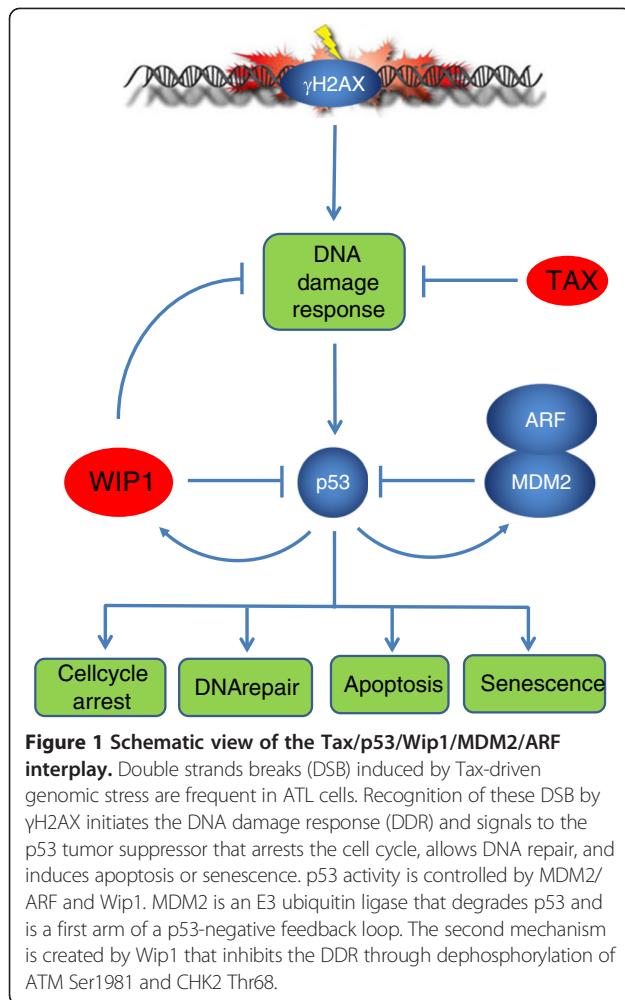
Wild-type p53-induced phosphatase 1

In the paper by Zane *et al.*, the predicted role of p53 was first validated in a transgenic mouse model, confirming previous reports. By crossing Tax-transgenic and p53 knock-out mice, they show that tumor-free survival is significantly shortened in a p53^{-/-} background. The authors next evaluated the role of Wip1 (Wild-type p53-induced phosphatase 1), a regulator of p53. Interestingly, Wip1 deficiency reduces Tax induced tumorigenesis in Wip1^{-/-} and Wip1^{+/-} mice. Consistent with their inhibitory activity, transient expression of Tax and Wip1 reduced p53 transcriptional activity in reporter assays. Although the mechanisms still need to be further characterized, a plausible model is that Tax and Wip1

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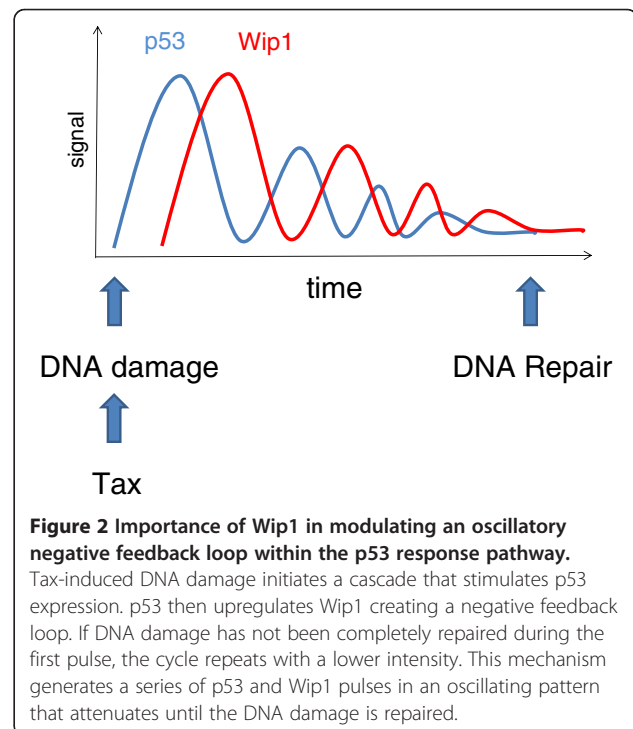
cooperate in tumorigenesis via p53 inactivation. The paper by Zane *et al.* thus extends previous observations showing resistance to transformation by other oncogenes such as Ras, Myc, E1A and Ebb2 in PPM1D deficient cells [10].

Wip1 is a PP2C family serine/threonine phosphatase that inhibits the function of several tumor suppressor pathways, including ATM, CHK2, p38MAPK and p53 [10]. PPM1D (protein phosphatase, Mg²⁺/Mn²⁺ dependent, 1D), the gene encoding Wip1, is aberrantly amplified in different types of human primary cancers. Conversely, deletion of PPM1D in mice decreases tumorigenesis. In breast cancers, p53 mutations are frequent, but tumors with PPM1D amplification rarely harbor p53 mutations. One explanation is that Wip1 promotes tumor formation through its ability to inhibit p53 tumor suppressor function directly or indirectly, thus reducing selective pressure for p53 mutations during the progression of cancer.

The paper by Zane *et al.* is important because it reveals the potential oncogenic role of Wip1 in Tax-mediated oncogenesis in a model of ATL. A series of open questions

remain regarding the biological relevance of Wip1 in patients and the mechanisms involved. First, it is currently unknown if gene amplification of PPM1D occur in ATL as observed in other types of cancers. Like p53 deletions [9,11], PPM1D genomic amplifications usually appear at late stages of tumorigenesis. Alternatively, it is possible that expression and/or activity of Wip1 are increased in the absence of genomic alteration. The PPM1D promoter contains at least two types of transcription binding sites: one for p53 that creates a negative feedback loop (Figure 1) and another for c-Jun [10]. Current data show that Tax, despite its ability to stimulate AP1, does not activate PPM1D expression in reporter assays. Moreover, Wip1 mRNA expression is not increased in Tax-positive cells. This observation needs to be further validated at the protein level using a larger number of HTLV-1 infected cell lines as well as in patients' samples. Confocal microscopy indicates that Tax and Wip1 colocalize in the nucleus. Further experiments will be required to determine direct or indirect binding between Wip1 and Tax. However, interactomic and proteomic analyzes currently suggest that both proteins do not physically interact (JC Twizere and OJ Semmes, personal communications).

In the context of HTLV-1 associated oncogenesis, it will be interesting to assess the role of other viral oncogenes like HBZ in the Tax/Wip1/p53 interplay. As an inhibitor of AP1 activity, HBZ might interfere with Wip1 expression. Combined crosses between Tax, Wip1, p53 and HBZ transgenic and knock-out mice could address this question. In this context, it is noteworthy that



deletion of p14(ARF), a MDM2 modulator (Figure 1), accelerates osteosarcoma formation further supporting the role of p53 regulation in Tax-induced oncogenesis [12]. ARF is a sensor of hyperproliferative signals such as those from the Ras and Myc oncoproteins. In response to oncogenic stress, ARF causes cell-cycle arrest in G1 and G2/M and is associated with increased p53 and p21 expression. ARF mediates cell-cycle arrest by directly binding to MDM2 and sequesters it in the nucleolus. Sequestration of MDM2 stabilizes and activates p53 which then blocks cellular proliferation. Although ARF does not appear to be aberrantly expressed in ATLL cells [9], acceleration of Tax-induced tumor formation in ARF-deficient mice further supports the central role exerted by p53.

Of particular interest is the Wip1-dependent regulation of ATM-CHK2 signaling on the dynamics of p53-dependent response to DNA damage [10]. In fact, recruitment of γ H2AX to double strand breaks initiates a cascade that produces different levels of p53 depending on the level of damage. This mechanism also initiates a series of p53 pulses in a repetitive pattern that shapes p53 protein level dynamics in an oscillating manner due to positive and negative feedback loops (Figure 2). If DNA damage has not been completely repaired during the first pulse, the cycle repeats with a lower intensity. MDM2 and Wip1 are two essential components of this feedback loop. Upregulation of p53 during the first pulse activates transcription of MDM2 and Wip1 which, in turn, can feedback negatively to p53 and ATM-Chk2, respectively. Disequilibrium in this intricate network could lead to incomplete DNA repair.

What are the outcomes of these observations in terms of therapy of ATL? First, Tax-repressed p53 function in HTLV-1-transformed cells is druggable and can be restored by treatment with 9-aminoacridine in a setting absent for p53 genomic alterations [13]. Secondly, arsenic trioxide, which has been recently proposed to prevent relapse of ATL lymphoma patients [14], augments Chk2/p53-mediated apoptosis by inhibiting Wip1 [15]. Thirdly, Wip1 can directly be targeted by specific inhibitors such as compound M (mercury, [4-Aminophenyl] [6-thioguanosinato-N7,S6]), CCT007093 ([2E,5E]-2,5-Bis [2-thienylmethylene]-cyclopentanone) or thioether cyclic phosphopeptide c (MpSIpYVA) [10]. Alternatively, phosphorothioate antisense oligonucleotides targeting PPM1 and specifically disrupting the binding of ATM-Wip1 or CHK2-Wip1 may be instrumental providing that delivery issues are solved.

Conclusion

In summary, the paper by Zane *et al.* adds a new piece in the complex puzzle of Tax-induced oncogenesis and reveals a potential new mechanism of p53 attenuation

during HTLV-1 pathogenesis opening interesting prospects for therapy.

Competing interests

The authors declare no competing financial interests.

Authors' contributions

NG, AC, PYB and LW wrote and approved the manuscript. All authors read and approved the final manuscript.

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