## **News and Commentary**

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## αTAT1: a potential therapeutic target in cancer?

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Cell Death and Disease (2016) 7, e2172; doi:10.1038/cddis.2016.46; published online 31 March 2016

a-Tubulin acetyltransferase 1 (aTAT1) and its homolog are major *a*-tubulin acetyltransferases conserved in a wide range of species.<sup>1</sup> In human cells, it catalyzes the reversible acetylation of a-tubulin at Lys40. Unlike a-tubulin deacetylases, HDAC6 and SirT2, which have multiple substrates,  $\alpha$ -tubulin is the major substrate of  $\alpha$ TAT1 identified so far except for its auto-acetylation. These three enzymes largely control the cellular acetylated a-tubulin (Ac-Tu) level, which has been suggested to be involved in modulating a variety of cellular functions, including microtubule dynamics, cell migration, and motor protein transport. Despite its role in cellular physiology, depletion of  $\alpha$ TAT1 did not noticeably affect the viability and growth in several organisms, such as the nematode Caenorhabditis elegans, zebra fish, and mice, although morphological defects were found at the tissue or cellular level.1-5

In human cell models, *a*TAT1 depletion was reported to delay ciliogenesis without affecting cell morphology or proliferation in immortalized retinal pigment epithelial cells of RPE-hTERT.<sup>1</sup> Nevertheless, our recent study published in *Cell Death Discovery*<sup>6</sup> compared the effect of three *a*TAT1-specific small hairpin RNAs (shRNAs) in human cervix cancer cell line HeLa with lung cancer cell line A549 and revealed that efficient *a*TAT1 downregulation can impair cell proliferation and actin architecture. Using the lentiviral system, we delivered shRNAs to establish stable *a*TAT1-downregulated cells. At about 72 h after delivery, we noticed increasing number of rounded, detached, and abnormally large-sized cells in the more efficient two shRNA-treated groups. Significant decrease in F-actin and focal adhesions were also observed in the most efficient shRNA-treated group.

On further monitoring of cell proliferation by time-lapse microscopy,<sup>6</sup> we found that detached cells were mainly the cells entering M phase that cannot pass metaphase. HeLa is much more susceptible than A549 in this aspect. Failure at the cytokinesis stage was also increased, usually producing multiploid cells. Over 80% of the control cells entered M phase within the first 36 h of observation, whereas *a*TAT1 downregulation increased the population sustained at interphase in both cell lines (Figure 1a). These characteristics are consistent with mitotic catastrophe,<sup>7</sup> which could be induced by agents that impair microtubule stability or DNA integrity.

It has been suspected for a long time that acetylation helps to promote the stability of microtubules. After the discovery of Ac-Tu in the green algae Chlamydomonas in 1985,8 its intracellular distribution has been widely studied using the monoclonal antibody 6-11B-1.9 Stable or long-lived microtubules, such as axonemes, and residual microtubules after microtubule-depolymerizing drug treatment are usually enriched with Ac-Tu. During cell division, the kinetochore microtubule is also acetylated shortly after its assembly. However, in vitro studies did not support the direct influence of aTAT1 or Ac-Tu on microtubule dynamics, suggesting a more complicated relation among them. In our results.<sup>6</sup> the lack of Ac-Tu did not noticeably affect the shape of mitotic spindle or chromosome alignment at the metaphase in both cell lines. Meanwhile, only marginal change in microtubule outgrowth speed and inter-kinetochore distance was observed. Therefore, it seems worthwhile to consider the possibility that aTAT1 also participates in other steps of spindle assembly checkpoint.

The mechanism connecting Ac-Tu and DNA repair was explained in a recent study.<sup>10</sup> Microtubule-targeting agents that promote microtubule depolymerization, such as vincristine, usually decrease Ac-Tu; on the contrary, agents that promote microtubule polymerization, such as paclitaxel, usually increase Ac-Tu. On monitoring the DNA damage marker y-H2AX, the Ser139 phosphorylated form of histone H2AX accumulated at the DNA double-strand breaks, Poruchynsky et al.<sup>10</sup> found that pretreatment with either vincristine or paclitaxel prolongs the decline of this marker after treatments with DNA-damaging agents. The authors suggested that this sustained y-H2AX level can underlie the change of microtubule integrity by vincristine or paclitaxel, thereby impairing the transport of DNA repair proteins on it. Our results demonstrated that aTAT1 downregulation increased y-H2AX, but not the other two DNA repair response markers, p-CHK1 and p-CHK2.6 It is worthy to further differentiate the role of Ac-Tu in the DNA repair response without drastically altering the microtubule integrity.

An intriguing observation that aTAT1 depletion-induced deficiencies can be partially rescued by expressing its mutant that cannot acetylate a-tubulin,<sup>3</sup> implying that aTAT1 can play other roles independent of its acetylation activity. On the other hand, multiple aTAT1 transcription variants are present in cDNA databanks. Most variants consist of a conserved N-terminus, which is sufficient to specifically acetylate a-tubulin, and a divergent C-terminus with functions largely

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**Figure 1** (a) Efficient  $\alpha$ TAT1 downregulation increases M-phase error and cells sustain at interphase in HeLa and A549 cells. Cell fate until the first round M-phase completion in time-lapse recordings was traced manually and divided into four groups: (1) entered M phase and error at metaphase or cytokinesis was observed, (2) entered M phase and produced two daughter cells, (3) detached before entering M phase, and (4) attached but not yet entering M phase. Time of the anaphase onset represents M-phase time point. This figure is also used in our work published in *Cell Death Discovery*<sup>6</sup> (doi:10.1038/cddiscovery.2016.6). (b) Suspected model of multiple  $\alpha$ TAT1 transcription variants were required to maintain different cellular functions through mechanisms partly independent of Ac-Tu

unknown. A recent study demonstrated that a mouse *a*TAT1 transcription variant binds *a*-adaptin, which is involved in clathrin-mediated endocytosis, via the C-terminal region, thereby promoting local *a*-tubulin acetylation.<sup>11</sup> Taken together, we suspect that multiple *a*TAT1 transcription variants are required to maintain different cellular functions through mechanisms partly independent of Ac-Tu (Figure 1b). Therefore, although Ac-Tu level was maintained in HeLa cells stably expressing an *a*TAT1 transcription variant after *a*TAT1 downregulation, this could not be enough to prevent the overall observed deficiencies.<sup>6</sup>

Endogenous Ac-Tu level has recently been linked to metastatic behavior in breast cancer<sup>12</sup> and *a*TAT1 has been reported to be critical in chemotaxis in the human breast cancer cell line MDA-MB231.<sup>11</sup> In our study, HeLa and A549 showed different susceptibilities during cell cycle stages after *a*TAT1 downregulation. To date, there is no clear evidence for the impact of mutations at *a*-tubulin Lys40 or *a*TAT1 on humans. Considering that *a*TAT1 depletion did not noticeably affect the viability in mice, differentiating the role of *a*TAT1 in more types of cancer and its potential as a therapeutic target are worthy of further investigation.

## **Conflict of Interest**

The authors declare no conflict of interest.

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