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Opponent's review of the doctoral thesis given by Mgr. Kseniya Ustinova entitled "Substrate specificity of histone deacetylases"

The submitted doctoral thesis by Kseniya Ustinova deals with the structure-function relationships of the histone deacetylase 6 (HDAC6), a unique Class IIb subclass of HDAC superfamily with two homologous catalytic domains. The enzymatic activity of HDAC6 involves the removal the acetyl group from specific lysine residues of many non-histone substrates in the cell cytoplasm and as such, it plays a major role in many cellular processes, such as cell migration, proliferation, signaling and stress or immune response. The first part of the thesis describes the determination of the HDAC6 enzyme specificity for tubulin proteins. The author demonstrated that HDAC6 recognizes preferentially the acetylated lysine residue (K40) on free tubulin rather than that on assembled microtubules and the HDAC6 deacetylation of microtubules occurs stochastically along the entire microtubule fibers. The second part of the thesis concerns the physical interaction between HDAC6 and tubulin showing that the deacetylation of free tubulin dimers both *in vitro* and *in vivo* is dependent on molecular recognition and ionic interactions between the positively charged residues in the N-terminal region of HDAC6 and negatively charged residues on the tubulins. The third part of the thesis is focused on the design, synthesis and structure-function characterization of a novel HDAC6 inhibitor (SS-208). This set of data is very interesting and important for the overall understanding of the biology of both HDAC6 and cytoskeleton.

The PhD thesis contains 101 pages (including the printed version of the articles) and it is conceptualized as a set of the three commented publications equipped with the Introduction section (26 pages), the Discussion and Conclusions section (5 pages), and the list of 174 references. The Results section is based on the results from three articles, which have been recently published in well-known international scientific journals. Indeed, the author of the doctoral thesis has the first authorship on one of the publications and she is the second and the third author of the other publications. The thesis is written in English, with minimum typographical or grammar errors. The text is logically constructed and well accompanied with the graphical support.

However, there are several points that should be commented:

- The Thesis is consistently focused on HDAC6, why the Thesis has a more general name „Substrate specificity of histone deacetylases"?
- The aims of the Thesis are not properly formulated.

- The numbering of the Figures in the Thesis is unclear. Each section has its own Figure 1, Figure 2, etc...It is better to use the numbering according to sections, i.e. Figure 1.1, Figure 2.1. This significantly confuses the Discussion and Conclusions part.
- Figure 1 (p 45) appears to be damaged.

Questions:

- Do HDAC6 (or other HDAC) undergo acetylations or other post-translational modifications, which could affect its (their) enzymatic activities?

- What is the mechanism of the deacetylation of the K40 residue in the α-tubulin subunit assembled in microtubules given the fact that K40 is located to the "cavity" (inner surface) of the microtubule fibers? Does HDAC6 reside in the cavity of microtubules during formation of the fibers? Is there any enzyme(s) or protein(s), which would specifically reside in the microtubule cavity?

- Does HDAC6 also regulate tubulin deacetylation and/or microtubules turnover in specific cell compartments, such as cilia in the polarized ciliated epithelial cells on mucosal surfaces?

- Does the N-terminal, disordered region of HDAC undergo a conformational change (fold) after binding to the tubulin dimers or interacts in an unfolded conformation?

- The SS-208 inhibitor has been shown to modulate antitumor immune response rather than to exert cytotoxic activity to cancer cells. Can you speculate about the molecular details of the SS-208 action?

In conclusion, I would like to note, that the results of the thesis are original and with significant scientific value. In my opinion, the thesis fulfils the requirements for obtaining the academic degree PhD and thank the author for the pleasure during the reading of her Thesis. I wish her success in her future research career and I recommend the submitted work as a basis for the defense.

In Prague, 14.9.2020

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