MUNI
MED

Brno, February 14th 2024

Opponent's opinion on the dissertation of Mgr. Veronika Antonyová
"Inhibition of TET-1 protein by iron chelators"

Student Mgr. Veronika Antonyová focuses on the complex characterization of TET-1 inhibitors, i.e. potentially promising targeted anticancer drugs. TET proteins are mainly involved in the process of DNA demethylation. Although the precise function of TET proteins in cancer development remains uncertain and logic may point to them as tumor suppressors, TET inhibitors could potentially prevent DNA demethylation at oncogene promoters, similar to how DNMT inhibitors reverse aberrant cancer hypermetylation.

The student demonstrated a rational approach to target TET not just because of literature (association between TET overexrpression, aberrant hypermethylation and worse overall survival). The TET proteins contain Fe(II) ions in their enzyme active centers. Iron chelators could therefore logically inhibit TET function. The author used a spectrum of approaches to characterize either the newly synthesized drugs and described interaction with the receptor protein and cells. The range of techniques is rationally chosen and adequate for the purpose.

In the introduction, the author logically describes the topic in a well-written and easy-to follow text. However, some parts of the introduction contain unrelated text, e.g. on histone modifications or non-coding RNAs. Next, the author clearly defines the hypotheses for the work, and in the results, the author presents them in a methodological order. There is a lack of information on the variability of the measured data, as well as a lack of statistics (at least in the sections where this is relevant, typically the cell line data). The presentation of results is difficult to follow, systematic visualization of the results together and pointing out what the results mean together is somewhat lacking - for example, the data indicate that the higher the binding log(K), the lower the IC50 for TET inhibition, but such information is difficult to see in the results and only briefly discussed in the conclusion or in the appended articles.

The author demonstrated strong capability to participate in the preparation of scientific articles. The presented results are mostly based on the result of one published WOS-indexed paper, in addition the author has published three other WOS-indexed papers relevant to this topic (one of which is a well-written review). The author is also author/co-author of four other papers, more than necessary to prove his ability to work in the scientific field.

Masarykova univerzita, Lékařská fakulta

Kamenice 753/5, 625 00 Brno, Česká republika
T: +420 549 49 2910, E: info@med.muni.cz, www.med.muni.cz

In conclusion, despite my comments on results presentation and interpretation I recommend the thesis for defense. The student has clearly demonstrated his or her creative abilities and the thesis meets the requirements for a dissertation.

Questions

1. There is a fairly clear negative relationship between binding affinity and IC50 for TET1 inhibition, the higher the binding log(K), the lower the IC50 for inhibition. Furthermore, the binding properties to TET1 (affinity) as described by KD were not correlated. And in comparison, binding energy did not correlate significantly with KD or IC50. How do you explain this?

2. The colocalization of chelators with mitochondria is interesting for at least several reasons. Krebs cycle metabolites act as TET inhibitors. Is there literature describing their docking scores? I find such information interesting in the context of the docking score of the tested inhibitors.

3. Although partly mentioned in the discussion of the attached article, can you comment more on the topic of potential effects of TET inhibitors on mitochondrial DNA methylation?

4. Why is the analysis of subcellular localization performed in fibroblasts, in which it is difficult to assess subcellular structures due to their morphology, when epithelial tumor lines were also tested, not to mention the fact that the IC50 was highest for fibroblasts and cancer cells are the primary target of the treatment?

doc. MUDr. Jaromír Gumulec, Ph.D.
Docent

MUNI
MED

Masarykova univerzita | Lékařská fakulta
Ústav patologické fyziologie,
Výzkumná skupina Michala Masaříka
A: Kamenice 753/5 | 625 00 Brno
T: 549 498 526
E: j.gumulec@med.muni.cz