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Re: Barbora Šalovská's dissertation thesis entitled: 'Proteomic analysis of gamma-irradiated human leukemic cells'.

To whom it may concern,

Hereby I submit my review of the doctoral thesis of Barbora Šalovská entitled 'Proteomic analysis of gamma-irradiated human leukemic cells'. The thesis is mainly divided into Introduction, Results, and Discussion. There are several smaller sections and an appendix of all previously published work. The work presented in this doctoral thesis spans a wide range of expertise: biological background of DNA damage / repair, systems pharmacology experiments, analytical chemistry method optimization, use of high mass accuracy mass spectrometers, computational primary proteomics data analysis, statistical significance analysis and most importantly data integration and interpretation.

Generally the thesis is written in an engaging style. In my experience, this was the most convincing argumentation linking single/double stranded DNA break to cell cycle arrest and cell death. Also, the technical review of chemical and metabolic labelling methods, phopho-proteomics enrichment methods and computational aspects of primary proteomics data analysis show the wide range of topics covered in this thesis. The experimental section features very polished final figures. From the yet unpublished work there are multiple avenues described in the Discussion section to interpret the systems biology data collected. I am looking forward to reading the final publication on the characterization of the phopsho-proteome and metabolome of ATR inhibited MOLT-4 cells in the future.

Some questions arose while reading the thesis, which may serve as discussion points during the thesis defence:

- On page 71, it states '20% Acn/NH4OH, pH 11.5'. What is the concentration (or percentage) of NH_4OH in the elution buffer?
- Figures 26 and 27 on pages 139 and 140, respectively, show metabolomic data of ATR inhibition focused on purine/pyrimidine metabolism. It is curious that only nucleotides show a decrease in abundance in 'IR + VE 12' samples, while nucleosides are increased. The following discussion correctly states that nucleotides provide an energy source for the cell.

Ribo-nucleotides are also used for transcription / translation. Is there any evidence that could support a protein synthesis increase in 'IR + VE 12' samples and therefore partially explain the low abundance of nucleotides?

- For me the most surprising finding was that the KU55933 inhibitor has a K_D in the low nano-molar range to ATM. However, even at 10 μM and IR the ATM inhibitor has only a small effect on treated MOLT-4 cells (Figure 11, page 89). Is this effect cell line specific or is the K_D exclusive for in vitro experiments?

In closing I would like to mention that insight into phopho-peptide enrichment presented in Barbora Šalovská doctoral thesis already made a positive impact on optimizing the phospho-peptide enrichment protocol in my own laboratory. Further, I thank the thesis committee for giving me the opportunity to review this thesis which I hereby review positively. I believe that the systems biology approach coupled to specific biological follow up experiments will be a great asset to Barbora Šalovská's future scientific career.

Kind regards,

Dr. H. Alexander Ebhardt.