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Cancer development is multistep process leading to cancer metastasis associated with spread of malignant cells via patient body. The systemic spread of tumor cells is the ultimate cause of most deaths from cancer, yet few successful therapeutic strategies have emerged to specifically target the process of cancer metastasis. Cytoplasmic linker associated proteins (CLASPs) support the morphological changes of malignant cells via metastatic spread. Thus, inhibition of CLASPs might prevent the cancer metastasis and might be also combined with other anti-cancer modalities for further clinical benefits.

Presented thesis aims to investigate the role of immunogenic cell death induced in CLASP depleted tumor cells by mechanical pressure. Moreover, study aim to investigate the potential impact of DAMPs release on macrophage activation and CXCL10 production, which might lead to migration of T cells and other effector immune components to the tumor microenvironment leading to development of immunologically hot tumors and improved response to immunotherapy.

The proposed scientific hypothesis is interesting and might be relevant if correctly addressed. However, presented thesis has several formal, scientific and language limitations. Introduction part is written inconsistently, with burdensome details in distinct parts and lack of important information in others. Due to language limitations some parts are confusing and would benefit from proof-reading and corrections, if the thesis might be shared for study purpose to other students. Regarding chapter order, I would suggest going from tumor immunology towards cancer metastasis and individual forms of cell death with its impact on anti-tumor immunity development and its relevance for cancer immunotherapy to guide our readers over complex and demanding topics. Finally, paragraph in chapter 2.2: “Therefore, hot tumor therapy is mainly multitargeting immune cells in terms of their activation, trafficking, infiltration, and antigen presentation in personalized cancer immunotherapy, whereas cold tumor therapy is focusing on transforming cold tumors into hot ones” is very confusing and I believe needs author comment or correction, since there is currently no official therapy for hot and cold tumors approved by FDA.

Result section does not apply standard requirements for scientific literature. Here, unfortunately, author combine introduction, results and discussion to present study findings. Results section must individually connect with each of

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the questions, which ensures clarity and minimizes confusion while reading, avoiding discussion of presented data. Current version of results does not apply to these principles. Moreover, results need to refer to respective figures with proper indication and statistical analyses, experiment conditions and graphs need to be label uniform across experiments. Recurrence of finding, including study limitation comments leads to reader confusion.

Finally, in discussion section author provide clear summary of available findings, including correct interpretation and mainly addressing all limitations related to study with potential application for future research. Taken together, this part proves that author not only understand presented findings and but is also able to discuss them in the context of available literature, which I highly appreciate.

Thus, despite numerous limitations of presented thesis, I would personally highlight several important points. Author performed this study over foreign internship. Thus, in limited time, he adopted novel scientific topic, novel methodologies and acquire some basic principles of scientific data interpretation and writing. Thus, I believe that the theses preparation was demanding and led to development of various skills which needs to be positively evaluated and highlighted here. Thus, despite my numerous critical comments and despite obvious need for further author improvement in scientific data interpretation and writing, I recommend the thesis for the defence.

I have several major comments/questions to the author:

1) The fundamental part of this thesis claim that ICD induced by mechanical pressure in CLASP depleted cells positively impact the activation of macrophages. However, I have several questions regarding these findings:

- Are there any data supporting the evidence that mechanical pressure induces ICD in this experimental setting? Which DAMPs are released by mechanically treated tumor cells?
- How was defined the intensity of experimental pressure used *in vitro*?
- DAMPs exposure and release according to available standards and literature needs to be evaluated on apoptotic cancer cells. Can you distinguish between apoptotic and necrotic cells within your *in vitro* experiments?
- Treated cell supernatant was used for co-culture experiments with THP1 cells. Is there any scientific rationale for this strategy? I believe the study would largely benefit from co-culture with entire tumor cells bodies to employ the panel of surface exposed and actively and passively released DAMPs.

2) Mechanically induced ICD was later compared to chemo-induced ICD. Are there any chemotherapeutics clinically available for melanoma therapy inducing ICD? Based on which criteria a modality can be claimed as immunogenic cell death inducer?

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3) CLASPs proteins are expressed in commercially available melanoma cell lines as shown in Figure 8. What´s the expression of CLASP proteins in primary melanoma tumor cells? These finding are fundamental for further clinical development.
4) Please, can you provide further details to comment on clinical relevance of your observations?
    a. How the CLAPSs proteins might be depleted from primary tumor cell for potential cancer therapy?
    b. Is there any available therapy under preclinical or clinical development targeting CLASPs?
    c. Is there any theoretical assumption about combination of CLASP depletion strategies with FDA approved immunotherapy?
5) As stated by author, THP1 cells do not provide all classical features of human macrophages. However, did you have a chance to evaluate any phenotypical markers of macrophage activation after co-culture with treated cancer cells? Can you briefly explain the role of macrophages in cancer?
6) Please, can you comment on term “hot tumor therapy” and “cold tumor therapy”, is there FDA approved immunotherapy only suitable for immunologically cold and hot carcinomas?

Minor:

1) I haven´t found figure 1D addressed in the text.
2) Can you please explain results presented in Figure 1b. Why melanoma cells without any type of treatment produce CXCL10? Why control shRNA group also produce CXCL10?

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