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Reviewer A

Siiskonen et al. reported that the rh-chymase was able to effectively detach primary tumor melanoma cells from the plastic surface in vitro and chymase+ mast cells can be found in apparent morphological contact with melanoma cells in vivo. They also showed that the rh-chymase at concentration range from 0.005 μg/ml to 0.01 μg/ml were able to decrease migration and proliferation in the cultured WM115 cell line of melanoma. On the other hand, they also claimed that that the viability rate in G361 cell line of melanoma was partially reduced by the presence of rh-chymase at 1-5 μg/ml. Then, they concluded that the effect of chymase may be antitumorigenic, rather than protumorigenic, since the mast cell chymase not only detach melanoma cells from the tumor, but also reduce proliferation, migration or viability of melanoma cells.

Major comments
I feel the design of this study was not reasonable and the molecular mechanisms of chymase relating the detachment, migration, proliferation and viability of melanoma cells were not examined at all. It is well known that human mast cell derived chymase able to generate many bioactive substances, such as angiotensin II, MMPs and TGF-bata1. From preset study, if the roles of chymase in the regulation of detachment, migration, proliferation and viability of melanoma cells were induced by the direct or via the enzymetic activated molecules is unknown. Concerning the design of the present study, they evaluated the chyamse actions at very different concentrations. For example, the migration and proliferation of melanoma cells were examined at the concentration of chymase range from 0.005 μg/ml to 0.01 μg/ml, while the viability of melanoma cells was examined at the concentration of chymase range from 1 to 5 μg/ml.

Reply: The authors agree with the reviewer that the molecular mechanisms of chymase actions was not studied here. The reason for that is that the current paper focused on finding out if chymase has any effects and can release adherent melanoma cells from the plastic surface and collagen-coated plastic surface. This aim was designed because in our previous study published in Melanoma Research in 2015 (ref

15 in the manuscript) we found that the reduced number of chymase-immunopositive cells has an association with microsatellites in melanoma tumors. We agree that the mechanism should be clarified, but it is a project of another publication. For example, chymase has the potential to degrade numerous extracellular matrix proteins and can cleave the basement membrane resulting in epidermis-dermis separation. Furthermore, chymase is able to activate procollagenase. Therefore, a variety of aspects needs to be clarified in further experiments, in addition to those suggested by the reviewer A.

The reviewer is right that very different concentrations of chymase were used in the paper. The concentration ranges were tested beforehand and those presented in the paper were found optimal for the experiments. Our previous publication "Diaconu NC, Rummukainen J, Naukkarinen A, Mättö M, Harvima RJ, Pelkonen J, Harvima IT. Mast cell chymase is present in uterine cervical carcinoma and it detaches viable and growing cervical squamous carcinoma cells from substratum in vitro. Arch Dermatol Res. 2011 Sep;303(7):499-512." (ref 18 in the manuscript) was used as a reference for the concentrations. In addition, we first performed the experiments for cell detachment. After those experiments, we chose lower concentrations for migration experiments, because we did not want the cells to detach during migration because of chymase. For viability tests, much higher concentration was used to really test the cells for possible toxic effects of chymase.

Changes in the text: To address this, we have added several new lines in Discussion, page 16 to highlight the need for further studies to investigate the effects of chymase in melanoma. In addition, to make the reason for the chosen concentrations clearer, we have added several new sentences in Methods, pages 7-9.

Minor comments

1, For the improvement of quality of present paper, it is necessary to provide clear microscopic photos reflecting the detachment, migration, proliferation and viability of melanoma cells.

Reply 1: The authors acknowledge this valuable comment and agree with the reviewer that such photos would make the manuscript clearer. However, we have used methods that provide only numerical data. For example, migration was counted from several images taken at different time points and the images as such are not informative. Proliferation was evaluated by ^{3}H-thymidine incorporation and measured by radioactivity and there are no photos available. For the cell viability, the case is similar. In figures 2 and 3, the cell detachment has been illustrated.

Changes in the text: None.

IMPACT FACTOR 0.496

2, Concerning the morphological contact between chymase+ mast cells and melanoma cells, authors should provide more high magnification photos or electron micrographs.

Reply 2: The authors thank the reviewer for this comment. We have modified Figure 1 to show cell contacts in higher magnification. However, with higher magnification less cells are shown. We decided to use the present magnification as it shows an area with several mast cells and melanoma cells. It is noteworthy that chymase is located in the secretory granules of mast cells and therefore can diffuse to a more distant site from the site of degranulated mast cell. Therefore, a true cell-cell contact may not be necessary for the action of chymase. Unfortunately, we don´t have access to electron microscopy.

Changes in the text: We have modified figure 1 and it now shows contacts with higher magnification. We have also added a new sentence ("Chymase is located in the secretory granules of mast cells and therefore diffuses to the surroundings from the site of degranulated mast cell.") in Results, page 10, line 234 to highlight the fact that chymase diffuses from degranulated mast cell.

Reviewer B

In this study, Siiskonen and Harvima evaluated the impact of recombinant chymase on cultured melanoma cells. They show that chymase causes detachment of melanoma cells from various substrates. It is also shown that chymase decreases the migration of one of the studied melanoma lines, and that chymase decreases its proliferation. These data are of potential relevance for the involvement of mast cells in melanoma. Moreover, the experimental approaches are sound, and the manuscript is overall well written.

Reply: The authors thank the reviewer for this comment.

Changes in the text: None regarding this comment.

Reviewer C

The study has the potential to be of general interest after corrections.

I am surprised that the authors having extensive pathological depository (151 tissue samples) have not performed an adequate analysis of this material in the context of

mast cell derived chymase.

This study is surprisingly underdeveloped and underreported. Unless there is no conclusive data on the role of enzyme or mast cells expressing the enzyme in evolution of melanocytic tumor. Even, negative data would be of interest if the studies are adequately performed.

First, provide information in the table on details of biopsies or excisions. Bening nevi (intradermal, compound, junctional...etc), dysplastic nevi (characteristic please), melanomas - subclassify, and stratify according to the pT also analyse other parameters that are part of synoptic reporting. Table should contain info on sex, age and anatomical site.

Having this information perform semiquantitative analysis. Th analysis may also include clinical data overall or diseases free survival if available for the Cancer registry. Such analysis will provide useful information for the reader.

Tests on melanoma cells, maintained for years in culture provide information on cell culture phenomena that may not be related to real melanoma behavior.

Also use presence or absence of melanin pigment as an additional variable in the analyses (Frontiers in Oncology 2022;12. DOI: 10.3389/fonc.2022.842496) and discuss.

As it stands, the conclusions based, on limited findings on two melanoma lines are not properly justified.

Clinico-pathological analysis has to be done

Reply: The reviewer is right that such detailed information and analysis of the pathological depository is very valuable. We have already published the clinico-pathological analysis of the samples in "Siiskonen H, Poukka M, Bykachev A, Tyynelä-Korhonen K, Sironen R, Pasonen-Seppänen S and Harvima IT. Low numbers of tryptase+ and chymase+ mast cells associated with reduced survival and advanced tumor stage in melanoma. Melanoma Res 2015 Aug 27;25(6):479-485". This is reference number 15 in our current manuscript and we site it in the introduction (pages 3 and 4), in Methods (page 5) and in Discussion (page 15). We hope this fulfills the request kindly raised by the reviewer.

In regard to melanin pigment, the stainings were evaluated for the previous paper (ref 15) and approximately 75 % of the histological samples contained melanin. Cell morphology was used to confirm correct cell types and when melanin pigment inhibited reliable evaluation of the sample, the sample was omitted from the analysis.

Changes in the text: To make it more clear, we have modified text on page 5, lines 110-113 and it reads now as "The 151 tissue samples consisting of 48 deeply (>4mm) and 39 superficially (<1mm) invasive melanomas, 14 in situ melanomas, 25 benign and 25 dysplastic nevi, collected at the Kuopio University Hospital as described with

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detailed clinico-pathological data in (15) were analyzed for apparent cell-to-cell contacts between chymase$^+$ mast cells and melanocytic cells."

Furthermore, in the end of Discussion, we emphasize the need to use several melanoma cell lines ("Our data highlight the need of using many different melanoma cell lines in studies in vitro to see the variation"), because apparently melanoma is very heterogenous tumor. To emphasize this better, we have added a new sentence with a new reference on page 16, lines 384 ("These results emphasize the heterogenous nature of melanoma (38)."). The new reference is (38) Shannan B, Perego M, Somasundaram R, Herlyn M. Heterogeneity in Melanoma. Cancer Treat Res 2016;167:1-15.