Reviewer's report

Title: Immunity to melanin and to tyrosinase in melanoma patients, and in people with vitiligo - possibility for modulation by appropriate diet?

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Reviewer: An M.T. Van Nuffel

Reviewer's report:

The article deals with the question whether diet e.g. mushrooms that carry the antigen tyrosinase and melanin, could contribute to the adaptive immune response in order to avoid melanoma relapse after resection of the primary tumor in patients without metastasis. Therefore, in the first instance they look whether and which type of humoral immunity is present in melanoma patients and compare therefore against vitiligo patients and healthy donors.

Major compulsory revisions
1) The authors mention in their manuscript that human and mushroom tyrosinase share some immunogenic epitopes. However, they do not indicate the exact percentage of homology between both proteins. Please include this in the manuscript.

Furthermore, they do not show whether the immunoglobulins found were directed against the shared epitopes or against epitopes specific for mushroom tyrosinase. I believe this is crucial information to estimate the protective potential of the present immunoglobulins against malignant melanoma. They could for instance screen with synthetic human tyrosinase as they did for melanin to assure that the presence of the immunoglobulins can contribute to the control of malignant melanoma.

2) The authors claim in the last sentence of the results section that the PBMC of healthy individuals proliferate better upon stimulation with melanin compared to the PBMC of melanoma patients. To me this is not visible in figure 4, panel A. Only in combination with PHA a slight increased proliferation is observed, which is likely due to the PHA. Therefore, this statement should be changed.

Minor essential revisions
1) Tyrosinase is a membrane associated glycoprotein that is directed towards the melanosomes as it is a key enzyme in the melanin synthesis. To which extend does it becomes expressed at the surface to allow opsonization by immunoglobulins? Also for melanin. What is its surface expression?

Please mention this in the manuscript.

2) If eating mushrooms could lead to humoral immunity against tyrosinase and melanin, it is important that the mushroom consumption habits of the patients in the three groups were the same in order to compare the presence and intensity
of this humoral immunity against these antigens. Is it checked that this habit is comparable in the three groups: the melanoma patients, the vitiligo patients and the healthy donors?

Please comment and include this in the manuscript

3) The authors mention that IgA would have a blocking effect, while IgM would be important to control the disease. Can they explain more in the discussion section why that could be? Are they sure that this is a direct effect of this isotype of immunoglobulin?

4) Throughout the figures, it might for some readers be difficult to see the difference between the red and bright red squares used to represent the data of the melanoma patients respectively with and without metastatic disease. Maybe it is an option to use open and filled squares?

5) PBMC is written fully for the first time in the material and methods section, while it is already used in the abstract and results section. Please write the full name the first time it is used. Also ADCC and MTT are only provided as an abbreviation. Please write this in full as well the first time the abbreviation is used.

Discretionary Revision

1) Is a minimal amount of mushrooms necessary to eat before humoral immune responses occur? Does the kind of mushrooms plays a role (e.g. brown mushrooms probably contain more tyrosinase and melanin)?

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests