Reviewer's report

Title: Eyebrow hairs contained the highest number of cutaneous human papillomaviruses from actinic keratosis patients

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Reviewer: Marisa Gariglio

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The manuscript by Ines Schneider et al. entitled “Eyebrow hairs contained the highest number of cutaneous HPV (beta and gamma-PV) in 75 patients surgically treated for actinic keratosis (AK) for whom the following samples were available: biopsies from AK lesions of the scalp, normal skin from non-exposed sites, and plucked eyebrow hairs. DNA from these specimens was tested for the presence of 28 cutaneous HPV (beta and gamma-PV) by a PCR based method.

A statistically significant higher number of cutaneous HPV infections were detected in 63/75 eyebrow hairs (84%) compared to 35/75 AK lesions (47%) and 28/75 normal skin (37%). HPV prevalence and the number of HPV types present in a single specimen were highest in eyebrow hairs compared to lower numbers in biopsies of AK lesions and normal skin. Concordant infections were determined with different stringencies which consistently indicated that the highest number of overlapping infections was observed in eyebrow hairs compared to AK or normal skin.

The manuscript is interesting because it shows thorough investigation of the cutaneous HPV infection patterns in different locations, either lesional (AK) or non-lesional (in both hair bulbs and normal skin) and, therefore, represents a step forward in understanding the natural infection of these skin-tropic viruses. The works is also strengthening the notion that eyebrow hair bulbs may be the most representative sampling for each individual HPV infection pattern. However, the manuscript suffers some criticisms that deserve further consideration as indicated below and cannot be published in the present form.

Minor Essential Revisions

Title. I suggest to modify it as follows “Eyebrow hair from actinic keratosis patients harbor the highest number of cutaneous human papillomaviruses”

1) The authors made some statements in the introduction claiming that beta 1 and beta 2 species display different pathogenic activity and reads “The molecular mechanisms of beta1PV (e.g., HPV5, HPV8, and HPV20) are different from beta2PV (e.g. HPV23 and HPV38). At present, HPV38 is the only cutaneous type, which is able to immortalize human primary keratinocytes indicating that the oncogenic potential of the beta2PV seems to be higher compared to the beta1PV
types.” Although some differences are emerging, I think we do not have enough evidence yet to fully support these statements. Based on that, I suggest to change 'are different' with 'may be different'.

Same for the second sentence… We do not actually know whether beta 2 species are more pathogenic than beta 1. I agree that HPV38 is the only beta type for which an in vitro transforming activity has been demonstrated, but, if we look at EV or more precisely the patients with primary immunodeficiencies (PIDs), beta 1 species have been primarily demonstrated in active infections. Based on this background, I would recommend to modify these sentences because this part of the introduction is also a bit controversial when compared to the second part mentioning EV skin cancer with beta 1 species and the same distribution of beta 1 and beta 2 found in other reports. While mentioning EV skin cancer, please cite more recent publications demonstrating transcriptionally active beta 1 infection in skin cancers (Dell'Oste et al JID 2009, and Borgogna et al Virology 2012).

2) The study population was already used for other purposes in a previous report (Nindl et al , B J Dermatol 2009). In this manuscript, as well as the previous one, it is not indicated whether the patients were immunocompetent or immunosuppressed. Since we are dealing with viruses which latently infect the host and can be reactivated under condition of immunosuppression, details about that must be included in the paper.

3) Figure 1 can be omitted because: i) prevalence of HPV infection is reported in the first paragraph of the results section, therefore, the graph is redundant and not necessary, and ii) concordance is reported in more details in Figure 4 and it is not clear (at least for this referee) about how it was calculated and represented in figure 1.

4) The discussion is a bit too long, most of it is dedicated to the comparison between the results obtained in this work and previous reports, and sometimes also a bit confusing. Because of interest, these comparisons should be expressed in a clearer way. I understand it won’t be easy, but efforts should be put on that, for instance including a table and describing better the results obtained first and then the comparison. For some cohorts from previous reports it is again not indicated whether they were from immunocompetent or immunosuppressed populations. As mentioned above, this is a crucial point which must be displayed and may be distinction between the two groups also help in making the comparison clearer and interesting for the reader. Another aspect that need to be discussed is the difference between AK and SCC samples, also since the precancerous AK lesions are known to harbor higher multiplicity of infection and higher viral loads in comparison to overt SCC.

Level of interest: An article of importance in its field

Quality of written English: Acceptable
**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' below