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Oral leukoplakia, human papillomavirus and cancer transformation

Akademisk avhandling

Som för avläggande av odontologie doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i sal Europa, konferenscentrum Wallenberg, Medicinaregatan 20, fredagen den 4e december 2020, klockan 13.00.

av

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Avhandlingen baseras på följande delarbeten

I. **Sundberg J**, Korytowska M, Miranda Burggos P, Blomgren J, Blomstrand L, De Lara S, Sand L, Hirsch JM, Holmberg E, Giglio D, Öhman J, Kovács A, Horal P, Lindh M, Kjeller G, Hasséus B. Combined testing of p16 tumour-suppressor protein and human papillomavirus in patients with oral leukoplakia and oral squamous cell carcinoma. *Anticancer Research* 2019; 39: 1293-1300.

II. **Sundberg J,** Öhman J, Korytowska M, Wallström M, Kjeller G, Andersson M, Horal P, Lindh M, Giglio D, Kovács A, Sand L, Hirsch JM, Araújo LM, Mamana Fernandes de Souza AC, Parlatescu I, Dobre AM, Hinescu ME, Henrique Braz-Silva P, Tovaru S, Hasséus B. High-risk human papillomavirus in patients with oral Leukoplakia and oral squamous cell carcinoma — A multi-centre study in Sweden, Brazil and Romania. *Oral Diseases* 2020; 00:1–10. DOI: 10.1111/odi.13510

III. **Sundberg J,** Korytowska M, Holmberg E, Bratel J, Wallström M, Kjellström E, Blomgren J, Kovács A, Öhman J, Sand L, Hirsch JM, Giglio D, Kjeller G, Hasséus B. Recurrence rates after surgical removal of oral leukoplakia – A prospective longitudinal multi-centre study. *PloS ONE* 2019: 14(12): e0225682.

IV. **Sundberg, J**. Pandey Dhakai S, Giglio D, Holmberg E, Kjeller G, Kovács A, Sand LP, Tokozlu B, Öhman J, Sapkota D, Hasséus B. Expression of p53, p63, podoplanin and Ki67 in recurring vs. nonrecurring oral leukoplakia. *In manuscript* 2020.

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Abstract

Oral leukoplakia (OL) is clinically diagnosed as a white oral lesion that cannot be scraped of or diagnosed as any other type of oral lesion. OL has the potential to transform into oral squamous cell carcinoma (OSCC). The gold standard treatment is a combination of surgical excision if possible, and surveillance. Despite complete removal, the risk of cancer or recurrence remains high. A major clinical problem is to predict which patients that have an OL that turns into OSCC. Infection with human papillomavirus (HPV), especially high risk (HR) HPV types has been attributed a role in cancer transformation of OL. HPV is a ubiquitous virus transmissible between humans and with a global variation in prevalence and known to cause cancer of the cervix uteri but also an important factor in oropharyngeal cancer. The overall aim of this thesis was to investigate the influence of HPV infection in OL, which clinical-, histopathological and treatment factors that affect the recurrence rate after surgical removal and to investigate molecular markers connected to cell proliferation and cancer transformation.

The thesis is based on five scientific questions addressed in four different studies:

Study I*: Is there a correlation between overexpression of the tumour suppressor protein p16 and high-risk HPV infection in leukoplakia and OSCC?* In this study the level expression of p16 in OL was assessed by immunohistochemistry and in the same group of patients OL tissue samples were analysed by Real-Time polymerase chain reaction (RT-PCR) for presence of twelve high-risk and two low-risk HPV types. In parallel, p16-expressing OSCC were analysed for the same HPV types.

Study II*: Has the prevalence of high-risk HPV in leukoplakia changed over time? Does the prevalence differ between Sweden, Romania and Brazil?* In contemporary and historical patient cohorts from the three countries OL samples were analysed with RT-PCR for 12 high-risk and two low-risk HPV types. In patients with cancer transformation of their OL tumour samples were also screened for presence of the same HPV types.

Study III: *Which clinical and anamnestic factors correlate with the recurrence of leukoplakia after surgical removal?* Patients with OL that were surgically removed comprised the study cohort. At inclusion anamnestic and clinical factors were registered together with results of the histopathological examination. Study subjects underwent follow-up visits at 3-6 months post-surgery according to the study protocol. Recurrence was defined as reappearance of OL at the primary lesion site.

Study IV: *Can expression of the cell proliferation biomarkers p53, p63, podoplanin and Ki-67 predict the recurrence of leukoplakia after surgical excision****?*** In this study patients with recurring vs non-recurring OL were compared regarding molecules known to be part of or influence cycle regulation. Immunohistochemistry was utilized and cell quantification was performed on digitalised images.

The results showed that:

- A high expression level of the tumour suppressor protein p16 is not a stable biomarker for presence of high-risk HPV in OL or OSCC **(Study I)**.
- High-risk HPV were found in low levels in Brazilian OL patients but in none of the Swedish or Romanian OL patients. Nor was any difference in HPV prevalence registered when comparing historical and contemporary cohorts. OSCC preceded by OL were all high-risk HPV negative **(Study II)**.
- The cumulative OL recurrence incidence after surgical excision was 45% after 4 years and 49% after 5 years. Non-homogeneous OL and use of snuff were significantly correlated with recurrence after surgical removal. Recurrence was also significantly associated with OSCC development at the primary lesion site **(Study III)**.
- In exploring if the biomarkers p53, p63, Ki-67 and podoplanin could predict recurrence after surgical treatment, p63 overexpression was identified to have a significant correlation to recurrence **(Study IV)**.

In summary, this thesis adds new knowledge regarding OL and HPV infection, surgical treatment outcome, cancer transformation and potentially useful biomarkers. But the thesis also points out future research avenues. Other HPV types than what have been investigated in this thesis may disclose a causative correlation between HPV, OL and cancer transformation. To date, surgical treatment of OL shows high recurrence rates, which points for a need for new techniques in determining surgical margins. This could be possible with emerging new non-invasive *in vivo* diagnostic tools. The potential of using a combination of biomarkers connected to cell proliferation as predictors for recurrence but also cancerous transformation is a *modus operandi* that in the future could be implemented in creating treatment decision algorithms for OL.

Keywords: potentially malignant oral disorders, recurrence, virus

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