Chromosome instability

VALORISATION ADDENDUM

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Early detection of head and neck cancer is of utmost importance for prognosis and preservation of function. In an ideal scenario, progressive premalignant lesions will be detected before malignant progression has taken place. Especially in the field of head and neck cancer, possibilities for early detection are given for a number of subsites: e.g. mucosal alterations of the oral cavity can be visualized at ENT-examination and mucosal alterations of the vocal cords can give early complaints of hoarseness. If mucosal alterations are detected, tissue biopsies will guide the clinician in the decision whether treatment, i.e. surgical excision or radiation therapy, is necessary. In case of malignancy there is no doubt about the need of treatment. However, in case of premalignant lesions that harbor a risk of malignant progression to head and neck squamous cell carcinoma (HNSCC), decisions are more complex. Until now, the risk of malignant progression for different precursor lesions remains unclear. Furthermore, histopathological classifications of these lesions are variable and greatly observer dependent.
The same is the case for resection margins of malignant head and neck tumors: not only (minimal) residual disease, but also groups of premalignant cells in resection margins of tumors can lead to an increased risk of (local) recurrence.

Reliability and clinical value of histopathological diagnosis

The tissue analysis of mucosal biopsies is generally performed by histopathological assessment. However, the in literature reported malignant progression risks for the different grades of dysplasia differ largely[1,2] and no internationally accepted guidelines exist for the therapeutic management of these precursor lesions.
Therefore, the studies in this thesis have been performed in order to 1) analyze the reliability of today's histopathologic classification systems in terms of inter observer concordance and prognostic information concerning malignant progression risk and 2) to identify additional markers of malignant progression in premalignant laryngeal and oral lesions. Furthermore, markers for tumor recurrence in resection margins of oral squamous cell carcinomas were investigated.
Our studies showed that the histopathological assessment of premalignant head and neck lesions on the basis of the existing histopathological classifications, leads to an inconsistent diagnosis, related to inter-observer variability. Furthermore, the WHO classifications hyperplasia, mild and moderate dysplasia do not discriminate between progressive and non-

progressive lesions. An improvement of the reliability can be achieved by the introduction of a two-grade scale modification of the used histopathological WHO-criteria, i.e. a division of premalignant lesions in “low-grade” (hyperplasia and mild dysplasia) and “high-grade” (moderate dysplasia).

Chromosome instability detection and its additional value above the available markers of progression

While the histopathological diagnosis of a premalignant lesion does not provide reliable prognostic information on the malignant potential of a lesion, the clinician may have difficulties in the decision-making on the therapeutic management of a lesion. Therefore, additional molecular markers for the discrimination of progressive lesions are needed to solve this issue. In this thesis, we applied chromosome instability (CI) detection as an additional marker of progression in premalignant laryngeal and oral head and neck lesions. Chromosome Instability detection was performed by Fluorescent in Situ Hybridization (FISH) on chromosome 1 and 7 centromere probes. CI was defined as the presence of copy number variations (CNVs), e.g. trisomy of polysomy of one or both chromosomes. We proved that CI detection can be performed by the count of aberrant cell nuclei in 100 nuclei, with a cut-off value of ≥10% aberrant nuclei (PAN). This approach improves the objectivity of the assessment as compared to histopathological assessment. We combined the information provided by the histopathological diagnosis, which was converted to a two-degree classification, with CI and immunohistochemically assessed protein overexpression of p53 and Fass Associated Death Domain (FADD). This lead to a multi-parameter progression model based on the previously mentioned markers, which improved the prognostic value compared to the routine histopathological assessment. This predictive model could be considered as a new tool for clinicians that need a more reliable tool for the discrimination of potentially progressive head and neck precursor lesions. An improvement of the counseling and therefore a more appropriate treatment of head and neck premalignancies will probably lead to a long-term decrease of patients morbidity and mortality, which is in general high in HNSCC.[3,4]

Clinical applications of CI detection and additional molecular markers

The main proposed molecular marker of this thesis is chromosomal instability (CI), defined as copy CNVs of chromosomes 1 and 7, which can be easily

assessed by dual target, centromere probe FISH. The relative low complexity of this detection method makes it very attractive for application in the daily clinical setting of ENT-surgeons, maxillofacial surgeons and other clinicians who encounter patients with potentially malignant head and neck lesions. CI detection by FISH can be implemented in a two-day diagnostic procedure and provides a quick and reliable diagnosis, especially if combined with a simplified, two-degree histopathological assessment scale and additional p53 /FADD immunostaining. The application of a quick CI-detection procedure on fresh-frozen intra operative tissue samples is in development.

Cost-effectiveness of the suggested new diagnostic model

CI detection by FISH and immunohistochemistry can be performed with relatively low costs especially if compared to other, more complex molecular detection methods.
There are no data available on the total costs related to the diagnostic procedure, treatment and follow-up of HNSCC patients. However, it is clear that the health-care related costs of a patient who is treated for HNSCC are high and often include a surgical procedure, hospitalization of the patient, radiation therapy, revalidation, (years of intensive) medical follow-up and sometimes specialized supportive extramural health care in case of progressive and non-curable disease. Secondary costs related to a short-term or long-term decrease of work-productivity should also be taken into account. A global comparison of the costs made in our clinic for the surgical treatment of a premalignant vs. a malignant oral lesion showed that the costs are approximately 10 times higher for the latter (i.e. 1700 euro vs. 17000 euro). The implementation of CI detection and immunostainings in the diagnostic procedure will lead to a slight increase of the diagnostic costs which, however, are non-significant as compared to the amount that can be potentially saved by the prevention of the development of HNSCC. However, yet to perform cost-effectiveness studies could provide more information on this issue.

Future perspectives- prospective study design

Today, there is no generally applied diagnostic and treatment protocol for premalignant head and neck lesions, except for lesions containing severe dysplasia or CIS (based on the WHO classification), which are in general treated by radical surgical (laser)excision or radiation therapy[5] Based on the results of this thesis, future research should focus on the development of a standardized, uniform and validated treatment protocol for premalignant head

and neck lesions. Currently, we are working on the development of a prospective, clinical multi-center study on the diagnostic and therapeutic approach of head and neck premalignancies with implementation of CI detection. Also patient related parameters such as the quality of life in relationship to potential loss of functionality (i.e. voice quality, swallowing) and discomfort after a performed surgical treatment as well as the cost-effectiveness of this new approach of head and neck premalignancies will be subject of investigation. This prospective study may finally lead to the definition of the optimal moment for (surgical) intervention in head and neck premalignancies.

Last but not least, it would be of great interest to perform further research based on the same principles, on other premalignant lesions found in the aero-digestive tract, for which comparable diagnostic issues do exist.

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