# Detection of methylated tumor markers in head and neck cancer and the tumor environment

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Head and neck cancers have a high recurrence rate. Alterations in gene methylation patterns promote cancer emergence and recurrence. In case of oncosurgery one hypothesis is that some cancers relapse due to incomplete resection combined with the fact that remaining cancer cells are undetected by standard histopathology. Referring to the field cancerization concept, HNSCCs are surrounded by genetic and epigenetic alterations in histologically normal-appearing tissue. The aim of this study was to analyze already established diagnostic DNA methylation markers (HOXA9, ZNF671, ZIC1, PAX6, ZNF833) in the tumor center and surrounding tissue of HNSCC samples.

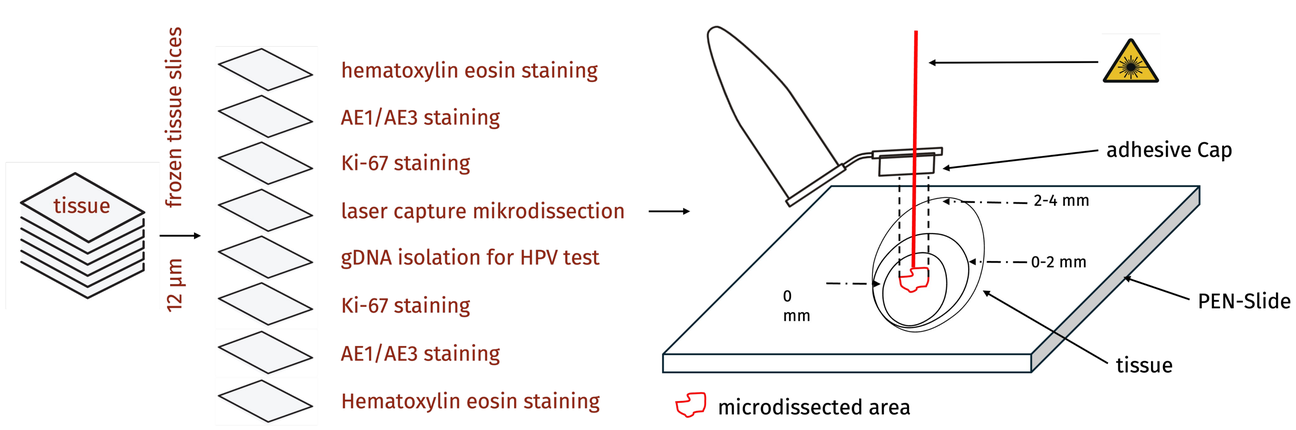


**Figure 1:** Sandwich method for staining an laser capture microdissection.

## Methods

Eight HNSCC samples (2x tonsil, 2x hypopharynx, 2x oropharynx, 1x oral cavity, 1x larynx) were collected during surgery and immediately frozen. Samples were processed according to the sandwich method (**Fig. 1**). Adjacent sections were used for immunohistochemical staining (Ki-67, AE1/AE3, HE). Sections in-between were placed on PEN-membrane slides used for laser capture microdissection (LCM) and HPV testing. Samples were collected starting at the primary tumor. While increasing the distance from the tumor samples of different tissue type were taken between 0 and 4 mm (stromal, epithelial and muscle cells)(**Fig. 2**). Methylation-specific real-time PCR (msPCR) was performed to analyze the methylation markers (HOXA9, ZNF671, ZIC1, PAX6, ZNF833).

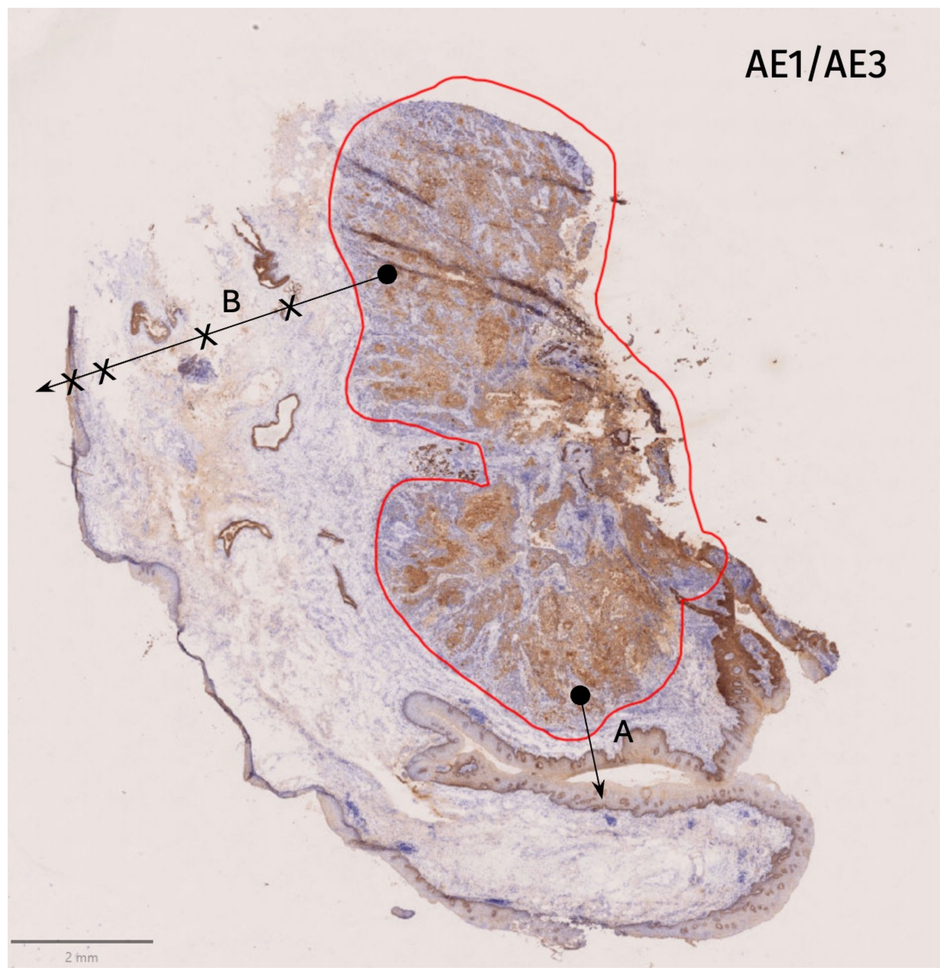


**Figure 2:** Example of distances of different microdissecten areas (X) to the primary tumor. Patient is HPV neg, tonsil cancer, TMN: cT4a, cN1, M0, collection: 26.01.2024

## Results

Every tumor marker, except ZNF833, possesses a detection rate greater than 77% in tumor cells. ZNF833 only has a tumor detection rate of 22.7% (**Fig. 3**). For the microdissected epithelial cells, ZNF671 has the highest detection rate of 33.3% while PAX6 has the lowest detection rate of 5.6%. ZIC1 and ZNF833 each have an epithelium detection rate of 11.1% and were not detected in stromal cells. While the other three tumor markers PAX6, HOXA9 and ZNF671 have detection rates in stroma cells, the percentages all fall below 6%.
Different tissue types were analyzed for malignant aberrations. As the distance from the primary tumor increased, no aberrations in ZIC1, ZNF833 and HOXA9 were detected. ZNF671 was found 0-2 mm and 0-4 mm from the tumor center, but its detection rate dropped from 11.6% to 3.6%, respectively (**Fig. 4**). PAX6 was only detected 0-2 mm from the tumor center (5.6%). The histopathological examination of the tissue types (stromal and epithelial cells) between 0 and 4 mm was classified as normal.

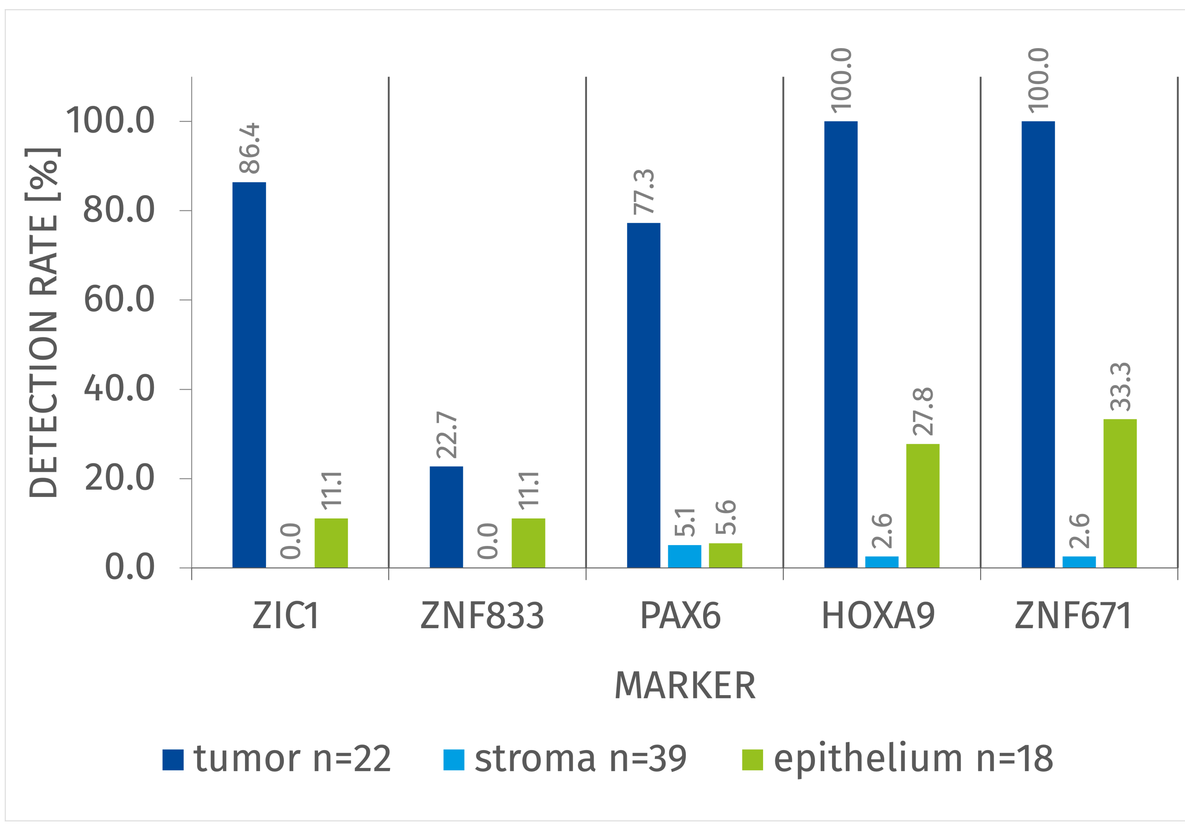


**Figure 3:** Detection rate [%] for each marker depending on the tissue type; n = total amount of samples taken from each tissue type of all eight patients.

## Conclusion & Outlook

In summary, the data shows that the markers are highly specific for tumor cells and less or not detected in normal stromal cells. Due to the fact, that ZNF833 is related to human papilloma infections it might explain the low detection rate. PAX6 and ZNF671 were detected at a distance between 0 and 4 mm from the primary tumor, which supports the field cancerization concept. As an outlook, it will be interesting to analyze whether a recurrence will occur in patients, the samples of whom had a positive methylation marker detection.

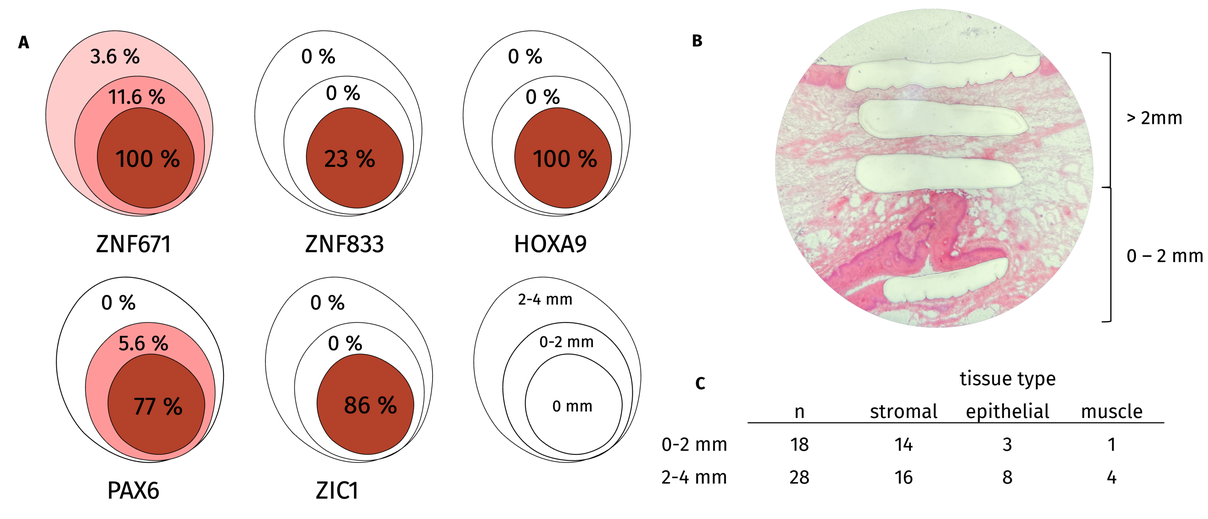


**Figure 4: A-C:** Detection rate [%] for each marker at the tumor center (0 mm), 0-2 mm from the tumor center and 2-4 mm from the tumor center (A), Example of microdissected areas between 0-2 mm and 2-4 mm; tumor centre not shown (B), tissue types between 0-2 mm and 2-4 mm (C).