Identification of BRAF Variant V595E in urine, smears and paraffin-embedded tissue: a new diagnostic tool for canine transitional cell carcinoma

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Introduction

Mutation of the BRAF gene
- Common cause of tumor formation in humans → Cellular proliferation and dedifferentiation
- Study of Mochizuki et al. (2015a+b) in the USA: BRAF variant V595E (syn. Y450E) in ca. 85% of the canine transitional cell carcinomas (TCC) (Plus ONE 2015a, 10(6):e0129534; Plus ONE 2015b, 10(12):e0141710)

Study Goals
- Examination of the prevalence of the BRAF Variante V595E in our routinely submitted samples
- Establishment of a diagnostic method for urine, cytological smears and tissue biopsies → Improvement of a non-invasive diagnostic method for TCC

Results

- Successful DNA-isolation from:
  - 39/45 Paraffin-embedded tissue
  - 38/48 Urine samples
  - 16/18 „Cell-rich“ cytological smears
  - 3/13 „Cell-poor“ cytological smears

Figure 1:
A: „Cell-poor“ cytological smear with solitary epithelial cells (arrow)
- the number of cells is too low for BRAF-Mutation analysis
B: „Cell-rich“, thick, cytological smear: epithelial cells can not be evaluated → typical indication for BRAF-Mutation analysis
C: „Cell-rich“, easily evaluable smear with transitional epithelial cells showing multiple criteria of malignancy

- Different samples:
  - Biopsy / paraffin-embedded tissue (A)
  - Urine (sediment) (B)
  - Cytology smears (C)

- After successful DNA-isolation, BRAF-analysis results were similar in different types of material originating from one dog

Table 1: Prevalence of the BRAF-gene mutation (BRAF Variante c.1784T>A) in the urinary bladder. Samples from 66 dogs (2/66: DNA-isolation was not sufficient)

<table>
<thead>
<tr>
<th>TCC (n=23)</th>
<th>Polyp (n=7)</th>
<th>Cystitis (n=23)</th>
<th>Other (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Homozogote Normal TT</strong></td>
<td>9</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td><strong>Reterozogote Variant TA</strong></td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion

- The BRAF-mutation analysis on routinely submitted samples (biopsies, cytological smears, urine) was established.
- **Requirement:** sufficient numbers of transitional epithelial cells in the submitted material is necessary.
- **Indications** for BRAF-mutation analysis:
  1) Non-invasive diagnostic method preferred
  2) Cytologically or histologically poor quality of material or questionable dysplastic appearance of epithelial cells
- The sensitivity of 70% in this study is corresponding to the sensitivity described in literature (67%-87%).
- The specificity is around 100%, since the BRAF-mutation was absent in all samples from dogs without TCC.
- **A positive result is confirming** the diagnosis of TCC (or possibly prostate carcinoma, which can also show this mutation).
- **Absence of the mutation** interpretation:
  1) Absence of a transitional cell carcinoma (for example: presence of a polyp or cystitis)
  2) Mutated transitional epithelial cells were absent in the submitted material (depending on sample size/representativity)
  3) The present TCC was not caused by a BRAF-gene mutation
- **A negative result does not rule out** the presence of a transitional cell carcinoma!
- **Prospect:**
  1) Use of the test for early detection of TCC in urine
  2) Prognostic and therapeutic relevance of the BRAF-mutation (for example: MAPKinase inhibitors)