Diagnostically relevant molecular alterations in salivary gland tumours

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Role of molecular testing

- Salivary gland neoplasms are a morphologically heterogeneous group
- diagnostically challenging
- discovery of tumor type-specific fusion oncogenes
- serve as diagnostic tools and in salivary cancer classification
- show promise as prognostic markers and targets of therapy
## Key molecular alterations in salivary gland carcinomas

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Chromosomal alteration</th>
<th>Gene fusion/rearrangement</th>
<th>Prevalence (%)</th>
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</table>
| (Mammary analogue) secretory carcinoma         | t(12;15)(p13;q25) t(12;X) | ETV6-NTRK3  
ETV6-RET             | 95-98 2-5            |
| Mucoepidermoid carcinoma                       | t(11;19)(q21;p13) t(11;15)(q21;q26) | CRTCI-MAML2  
CRTC3-MAML2       | 40-80 5            |
| Hyalinizing clear cell carcinoma                | t(12;22)(q21;q12)     | EWSRI-ATF1               | 80-90           |
| Adenoid cystic carcinoma                       | t(6;9)(q22-23;p23-24) t(8;9) | MYB-NFIB  
MYBL1-NFIB           | 25-80 10-20          |
| Polymorphous adenocarcinoma                    | 14q12                  | Hotspot activating PRKD1 somatic point mutation (E710D) | 20             |
| Cribriform adenocarcinoma of minor salivary glands | t(1;14)(p36.11;q12) t(X;14)(p11.4;q12) | ARID1A-PRKD1  
DDX3X-PRKD1  
PRKD2 and PRKD3 rearrangements | 24 13 16            |
| Salivary duct carcinoma/ IC                    | 17q21.1 3q26.32 inv(10)(q11.21q11.22) | HER2 amplification  
PIK3CA mutation  
NCOA4-RET           | 20-40 20 <5          |
Secretory carcinoma (MASC)
Mucoepidermoid carcinoma
Adenoid cystic carcinoma
Hyalinizing clear cell carcinoma

2A

2B

EWSR1

ATF1
Polymorphous adenocarcinoma (PAC) - WHO 2017 terminology, previously PLGA

- PAC-classic variant (PLGA)
- PAC-cribriform variant (CATS)

Hot spot point E710D mutations in PRKD1 gene

Translocations involving the PRKD1-3 genes
Salivary duct carcinoma

*HER2* gene amplification, mutations of *TP53*, *PIK3CA*, *PTEN* and *HRAS* NCOA4-RET fusion
Mucoepidermoid carcinoma

- Highly variable clinical prognosis
- Translocation \( t(11;19) \) fuses \( MECT1 \) (\( mucoepidermoid carcinoma translocated-1 \)) at 19p13 (also known as \( CRTC1 \)) with \( MAML2 \) (\( mastermind-like gene family \)) at 11q21
- Fusion positive patients have better outcomes
  - Less local recurrences, metastases and tumor-related deaths
Impact of CRTCl/3-MAML2 fusions on histological classification and prognosis of mucoepidermoid carcinoma

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Figure 1. Prognostic analysis for overall (A) and disease-free (B) survival of patients with mucoepidermoid carcinoma.

- Fusion positive cases of MEC have better outcome even in high grade morphology
Significance of MAML2 status in mucoepidermoid carcinoma

• Roughly 40-80% of tumors are characterized by CRTC1/MAML2 translocation, t(11;19)(q21;p13)
• About another 5% by CRTC3/MAML2 translocation, t(11;15)(q21;q26)
• these translocations were initially not seen in high-grade aggressive tumors
• Represent independent favourable prognosticicators
Significance of MAML2 status in mucoepidermoid carcinoma

- Recently, CRTC1-MAML2 translocations detected in a significant proportion of high-grade tumors
- Subsequent series failed to show significant correlation between MAML2 translocation status and outcome
- Positive translocation status does not guarantee indolent behavior
- Translocation negative tumors may not represent MEC
MAML2 Status in Mucoepidermoid Carcinoma Can No Longer Be Considered Prognostic Marker

To the Editor:

• MAML2 translocation status is diagnostic rather than prognostic marker!!!

Seethala, Chiosea. AJSP 2016;40:1151-3
Adenoid cystic carcinoma

MYB-NFIB fusion
Adenoid cystic carcinoma

- both minor and major SG, fairly common in sinonasal tract
- Highly infiltrative biphasic growth pattern of abluminal myoepithelial and luminal ductal cells
- relentless clinical course with late recurrences and distant metastases
- causes significant morbidity
- Perineural and intracranial invasion
Adenoid cystic carcinoma

- t(6;9)(q22-23;p23-24) MYB/NFIB in AdCC of both head and neck (salivary, lacrimal, ceruminal glands) and breast
- Translocation fuses MYB oncogene with transcription factor gene NFIB
  - Leads to chimeric MYB-NFIB fusion transcript
  - MYB activation through gene fusion is a major oncogenic event in AdCCa of many sites
FISH analysis in AdCCa

Fluorescence in situ hybridisation on FFPE sections using break apart rearrangement probe – ZytoLight SPEC MYB Dual Color Break Apart Probe  (6q23.3)

negative MYB break-apart  positive MYB break-apart
FISH analysis in AdCCa

- Fluorescence in situ hybridisation on FFPE sections using MYB/NFIB Dual Fusion probe
- Fused yellow signals are seen supporting presence of translocation
RT- PCR and nucleotide sequence analysis

- Sequence analysis of MYB/NFIB fusion transcript
Significance of MYB status in AdCC

- Fusion transcript MYB-NFIB was detected in more than 86% of our cases of AdCC, if all three probes are used
- **MYB-NFIB** is a robust [diagnostic marker](#)
  - Solid (basaloid) variant AdCC
  - Diagnosis of distant metastases
- Subset of AdCCa negative for **MYB-NFIB** (less 15%)
- t(8;9) translocation resulting in novel **MYBL1-NFIB** translocation
Secretory carcinoma (mammary analogue)

ETV6-NTRK3
ETV6-RET (ETV6-X)

Secretory Carcinoma (mammary analogue) MASC

Low grade vesicular nuclei, centrally located nucleolus, pink vacuolated cytoplasm
Mammary Analogue Secretory Carcinoma of Salivary Glands with High-grade Transformation

HG-MASC with atypical ETV6/NTRK3 fusion
Mammary Analogue Secretory Carcinoma of Salivary Glands with High-grade Transformation

Molecular Features of SC

- Most SCs harbor the recurrent balanced $t(12;15)$ (p13;q25) chromosomal rearrangement resulting in the fusion of the ETV6 and NTRK3 genes.
- The ETV6 gene split visualized by FISH.
- The classical $ETV6$-$NTRK3$ fusion transcript (exon 5-exon 15 junction) is in some cases not detected by standard reverse-transcriptase polymerase chain reaction (RT-PCR).
- Atypical fusion transcripts (4-14 or 5-14 junctions).
- $ETV6$-$RET$ fusion.
Sequence analysis of *ETV6-NTRK3* fusion transcripts in MASC
- 25 cases FISH ETV6 positive, RT-PCR negative
- Nested RT-PCR positive in 4
- 16/25 rearrangement of NTRK3
- 4 cases of MASC ETV6-X gene fusion
Secretory carcinoma with \textit{ETV6-X} translocation

MASC with \textit{ETV6-X} fusion, predominantly macrocystic and papillary structures
Secretory carcinoma with *ETV6-X* translocation

SC with prominent hyalinization and trabeculae of neoplastic cells embedded in a completely hyalinized central part
Molecular profiling of mammary analogue secretory carcinoma revealed a subset of tumors harboring a novel *ETV6-RET* translocation: report of 10 cases

- The presence of *ETV6-RET* fusion in SC was proved by at least three independent tests, i.e. NGS, FISH, RT-PCR
- Histomorphology is variable
- SC patients with *ETV6-RET* fusions may benefit from RET-targeted therapy
Secretory carcinoma with novel 
*ETV6-RET* translocation

SC with typical morphology and immunoprofile harboring a novel *ETV6-RET* translocation (4 cases)
Secretory carcinoma with novel ETV6-RET translocation

predominantly multicystic growth pattern with multiple mural nodules (3 cases)
Secretory carcinoma with novel ETV6-RET translocation

prominent fibrosclerotic stroma with isolated tumor cells in small islands or trabeculae (3 cases)
Secretory carcinoma- molecular testing

• SC has distinctive morphology and immunoprofile in most cases
• Diagnosis of „classical“ SC features could be performed without molecular testing
• However, in cases that depart from the typical features of SC in some way
• Molecular testing in SC is of potential value in treatment of patients
Treatment of SC

- treatment of SC has varied, ranging from simple excision to radical resection, neck dissection, adjuvant radiotherapy, and/or adjuvant systemic chemotherapy
- For patients presenting with a locally advanced, recurrent or metastatic disease the treatment options are currently limited and mainly palliative
- Testing for ETV6-NTRK3 translocation-pan TrK inhibitor Entrectinib (Ignyta) targets NTRK, ROS1, and ALK fusions
- ETV6-RET testing

Hyalinizing clear cell carcinoma of minor salivary glands

EWSR1-ATF1
Hyalinizing clear cell carcinoma of minor salivary glands

• HCCC is a rare salivary gland malignancy with squamous differentiation and prominent clear cell morphology
• WHO 2017- „clear cell carcinoma“
• recurrent t(12;22)(q13;q12) chromosomal translocation, leading to fusion of the EWSR1 and ATF1 genes
  o EWSR1 gene intact in myoepithelioma, PLGA, MEC, or epithelial-myoepithelial carcinoma
  o EWSR1 gene rearrangement detected in clear cell odontogenic ca and subset of myoepithelial ca with clear cell morphology

(Hyalinizing) Clear Cell Carcinoma

neoplastic nests and lobules are surrounded by, or admixed with a hyalinized basement membrane-like material.
(Hyalinizing) Clear Cell Carcinoma

- P63 protein
(Hyalinizing) Clear Cell Carcinoma

Dual stroma (BM-like and fibrocellular stroma)
Significance of testing of *EWSR1-ATF1*

- HCCC does not always show hyalinization or prominent clear cell differentiation
- Mucinous differentiation is not an exclusion criterion for HCCC
- HCCC has squamous differentiation and can mimic SCC
- It is distinct from EMCa, MEC, SMET and other clear cell and low-grade tumors of the head and neck (salivary, mucosal and skin) except CCOC.
Differential dg. HCCC versus MEC

- Milchgrub described a minority of cases with „occasional“ intracytoplasmic mucin
- HCCC has squamous differentiation
- these features overlap with MEC
- translocation $CRTC1/CRTC3$-MAML2 in MEC
- HCCC, with or without mucin, is negative for MAML2 and is distinct from MEC
- This impacts on grading and treatment as most HCCCs would be high-grade using MEC grading systems
EWSR1 rearranged clear cell myoepithelial carcinoma

- 25/72 (35%) cases of CCMC showed rearranged EWSR1
- Prognosis of CCMC poor
  - Died of cancer - 38%
  - Alive with recurrent/metastatic disease - 14%
  - Distant metastases - 33%
  - Lymph node metastases - 24%

FISH analysis using EWSR1 dual color, break apart probe
EWSR rearranged CCMC
EWSR1 rearranged clear cell myoepithelial salivary carcinoma
Clear cell malignant myoepithelioma of the salivary glands

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5 cases in major salivary glands
Significance of molecular testing for *EWSR1* in CCMC

• We described for the first time *EWSR1* gene rearrangement in a subset of MC arising in minor and major salivary glands.

• The *EWSR1* rearranged CCMC represent distinctive variant composed predominantly of clear cells with frequent necrosis and (most likely) more aggressive clinical behavior.

• Most *EWSR1*-rearranged CCMC of salivary glands are characterized by poor clinical outcomes.
Polymorphous adenocarcinoma (PAC) Significance of molecular testing

PAC-classic variant (PLGA)

- hot spot point E710D mutations in \(PRKD1\) gene

PAC-cribriform variant (CATS)

- translocations involving the \(PRKD1-3\) genes
Polymorphous adenocarcinoma (PAC)- classic variant

hot spot point E710D mutations in PRKD1 gene, no alterations seen in PRKD2-3 genes
Polymorphous adenocarcinoma (PAC)-cribriform variant (CASG)

translocations involving the PRKD1-3 genes (fusion genes included ARID1A and DDX3X)
Polymorphous adenocarcinoma (PAC) 
(WHO 2017 terminology, previously PLGA)

- Hotspot activating E710D point mutations in PRKD1 were reported in nearly three-quarters of PAC-classic variant (PLGA) cases
- Mutations in PRKD2 and PRKD3 were not found in PAC-classic variant (PLGA)
- Translocations involving the PRKD1-3 genes PAC-cribriform variant (CASG)
- Although CASG and PLGA have molecular alterations of the PRKD gene family, there are notable differences
Key Features distinguishing PLGA versus CASG

- PLGA is a low-grade infiltrative malignancy with a mixture of tubular, cribriform, papillary, and solid growth, arranged in fascicles with targetoid neurotropism.
- CASG is a tumor with distinctive cribriform/papillary glomeruloid patterns and highly vesicular papillary thyroid carcinoma-like nuclei predominating in base of tongue.
- PLGAs are characterized by PRKD1 E710D mutations, whereas CASGs are characterized by PRKD1-3 translocations.
- CASGs have a high capacity for nodal spread.
Salivary duct carcinoma

- SDC is a primary, high-grade salivary gland adenocarcinoma, characterized by morphologic features akin to high-grade ductal carcinoma of the breast
- About half of the cases arise from preexisting PAs
  - PLAG1 and HMGA2 alterations have been described also in SDC (SDC-ex-PA)
- SDC with apocrine phenotype almost uniformly expresses AR - 67-83%
- HER2 gene amplification is found in 20-30%
- Mutations in TP53, PIK3CA, and HRAS, and loss or mutation of PTEN
- NCOA4-RET fusion have been found in two SDCs
Salivary duct carcinoma

- HER2/neu and AR overexpression
Molecular testing in dif.dg. and treatment of SDC

- PLAG1 and HMGA2 oncogenes are specific for benign pleomorphic adenomas and CAex PA
- Detection of AR expression by IHC is a useful diagnostic aid in resolving the differential diagnosis with other high-grade carcinomas
- Androgen-deprivation therapy alone or in combination with conventional radiotherapy in some patients
- Treatment with anti-HER2 therapy in combination with bevacizumab and chemoradiation
Conclusions

• translocations and fusion oncogenes in salivary gland pathology
• clinically and pathogenetically relevant
• tumor-type specific
  o important in differential dg
  o prognostic biomarkers
  o development of therapeutic targets
Thank you for attention

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