**HER2 and EGFR Gene Copy Number Alterations are Predominant in High-Grade Salivary Mucoepidermoid Carcinoma**

Takafumi Nakano, MD; Hidetaka Yamamoto, MD, PhD; Kazuki Hashimoto, MD, PhD; Toshimitsu Nishijima, MD; Ryuji Yasumatsu, MD, PhD; Torahiko Nakashima, MD, PhD; Yoshinao Oda, MD, PhD; Shizuo Komune, MD, PhD

1. Department of Otolaryngology and Head and Neck Surgery, 2. Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University

---

**Abstract**

**Aims:** We aimed to investigate the molecular mechanisms underlying the development of Mucoepidermoid carcinoma (MEC).

**Methods & results:** We investigated the MAML2 fusion status using reverse transcriptase-polymerase chain reaction, and HER2 and EGFR status using immunohistochemistry and chromogenic in-situ hybridization in 31 cases. MAML2 fusions were detected in 57.7% MECs analyzed, including 68.8% low-grade, 50% intermediate-grade and 33.3% high-grade MECs. HER2 gene amplification was present in 14.3% MECs analyzed, including 25% intermediate-grade and 42.9% high-grade MECs. An increased EGFR gene copy number was detected in 14.3% MECs analyzed. Irrespective of MAML2 fusion status, all of 7 high-grade MECs had an increased gene copy number of either HER2 or EGFR gene in a mutually exclusive manner, whereas such abnormalities were extremely rare in low- and intermediate-grade MEC.

**Conclusions:** These results suggest that HER2 or EGFR gene abnormality may play an important role in the development of high-grade MEC, and also in the progression from MAML2 fusion-positive low/intermediate-grade to high-grade in a subset of MEC. Furthermore, we suggest that high-grade MEC consists of a heterogeneous group of tumors in terms of molecular pathogenesis in particular MAML2 fusion status.

---

**Introduction**

Mucoepidermoid carcinoma (MEC)

- **Histological features and grading**
  - Cell types: mucous (goblet) cells, intermediate cells, squamoid cells
  - Histological grade: low, intermediate, high
  - Histological grading parameters: cystic components, membranous invasion, anaplasia, four or more mitoses per 10 HPF
  - Prognosis

<table>
<thead>
<tr>
<th>Grade</th>
<th>5-year OS</th>
<th>5-year DFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>93%</td>
<td>88%</td>
</tr>
<tr>
<td>Intermediate</td>
<td>40-50%</td>
<td>20-40%</td>
</tr>
<tr>
<td>High</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Molecular alterations**

CRTC1 and CRTC3-MAML2 fusion genes

CRTC1-MAML2 fusion transcript: 30-80%

CRTC3-MAML2 fusion transcript: 6%

These fusion gene transcripts are almost detected in low- or intermediate-grade MEC, and associated with indolent clinical course.

- **HER2 and EGFR**
  - HER2 protein overexpression
  - HER2 gene amplification
  - EGFR protein overexpression
  - EGFR gene high-polyomasy

**HER2 protein overexpression**

- Low: 0/18 (0%)
- Intermediate: 1/5 (20%)
- High: 3/8 (37.5%)

**HER2 gene amplification**

- Low: 0/17 (0%)
- Intermediate: 1/4 (25%)
- High: 3/7 (42.9%)

**EGFR protein overexpression**

- Low: 10/18 (55.6%)
- Intermediate: 3/5 (60%)
- High: 5/8 (62.5%)

**EGFR gene high-polyomasy**

- Low: 0/17 (0%)
- Intermediate: 0/4 (0%)
- High: 4/7 (57.1%)

Squamoid, intermediate, mucous cells of low-grade MEC are negative for HER2 IHC (A), and contain each two signals of HER2 and CEP17 per nucleus, indicating no amplification (B). In high-grade MEC, tumor cells show membrane staining for HER2 IHC (C), and contain multiple HER2 signals per nucleus, indicating amplification (D).

**3. IHC and CISH**

**HER2 protein overexpression**

- Low: 0/18 (0%)
- Intermediate: 1/5 (20%)
- High: 3/8 (37.5%)

**HER2 gene amplification**

- Low: 0/17 (0%)
- Intermediate: 1/4 (25%)
- High: 3/7 (42.9%)

**EGFR protein overexpression**

- Low: 10/18 (55.6%)
- Intermediate: 3/5 (60%)
- High: 5/8 (62.5%)

**EGFR gene high-polyomasy**

- Low: 0/17 (0%)
- Intermediate: 0/4 (0%)
- High: 4/7 (57.1%)

Squamoid, intermediate, mucous cells of low-grade MEC are negative for HER2 IHC (A), and contain each two signals of HER2 and CEP17 per nucleus, indicating no amplification (B). In high-grade MEC, tumor cells show membrane staining for HER2 IHC (C), and contain multiple HER2 signals per nucleus, indicating amplification (D).

---

**Materials and Methods**

- **Case Materials**
  - 31 cases of MECs (formalin-fixed paraffin-embedded)

- **Methods**
  - 1. RT-PCR for MAML2 fusion
  - 2. Immunohistochemical staining (IHC) for HER2 and EGFR
  - 3. Chromogenic in-situ hybridization (CISH) for HER2 and EGFR

---

**Conclusion**

- HER2 or EGFR gene alterations may play an important role in development of high-grade MEC.
- Molecular-targeted therapy against HER2 and EGFR might be a promising therapeutic strategy for high-grade MEC.

- The possible pathogenesis and progression model of MEC
  - High-grade MEC may arise through three different pathogenic processes.
    - MAML2 fusion positive low/intermediate-grade MEC obtains either HER2 or EGFR gene number alterations, and progress to high-grade MEC.
    - MAML2 fusion negative low/intermediate-grade MEC obtains either HER2 or EGFR gene number alterations, and progress to high-grade MEC.
    - High-grade MEC having either HER2 or EGFR gene number alterations may occur ‘de novo’.

---

**Summary, Discussion & Conclusions**

- HER2 or EGFR gene alterations may play an important role in the development of high-grade MEC.
- Molecular-targeted therapy against HER2 and EGFR might be a promising therapeutic strategy for high-grade MEC.