Aberrant nuclear expression of GSK-3β in human head and neck carcinoma

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ABSTRACT

Background. Recurrent/metastatic head and neck squamous cell carcinoma (SCCHN) and salivary gland malignancies are difficult to treat with limited standard of care options at the present time. Glycogen Synthase Kinase-3beta (GSK-3β), a serine/threonine protein kinase, has been implicated as a potential therapeutic target in human cancer. Our in vivo studies demonstrated that our novel GSK-3 inhibitors significantly potentiated the effects of conventional chemotherapy in patient-derived xenograft models of glioblastoma and breast cancer leading to regression of tumors. In order to develop a rationale to test our novel GSK-3 inhibitors in head and neck (H&N) malignancies, we evaluated the expression pattern of GSK-3β in human H&N benign tissue and malignant tumors.

METHODS. We used immunohistochemical staining of H&N tumor tissue Microarray (TMA), 48 total cases (20 benign tissues, 28 malignant), to study the expression pattern of GSK-3β. GSK-3β nuclear accumulation was defined as positive staining of more than 50% of cancer cell nuclei throughout the tumor regardless of cytoplasmic staining.

RESULTS. There were total of 28 malignant H&N samples (22 non-salivary and 6 salivary origin). Of the 22 non-salivary malignant H&N samples (15 SCCHN, 2 nasopharyngeal and 5 other histology), 15 (68%) were found to have aberrant nuclear accumulation of GSK-3β. Amongst SCCHN, 73% (11 of 15 samples) had aberrant nuclear accumulation of GSK-3β. In contrast, none (0%) of the 11 benign non-salivary H&N tissue showed detectable expression GSK-3β. Of interest, 60% of salivary adenoid cystic carcinoma (ACC) specimens and 44% of benign salivary gland tissue showed GSK-3β expression.

Conclusions. Our results demonstrate that there is aberrant nuclear expression of GSK-3β in SCCHN. This finding supports the clinical exploration of GSK-3β inhibitors in SCCHN and further study of GSK-3β as a potential prognostic and predictive biomarker for risk of recurrent disease and chemotherapy resistance in patients with advanced SCCHN. The role of aberrant nuclear expression of GSK-3β in salivary gland malignancies merits further study.

OBJECTIVES

- Develop a rationale to test our novel GSK-3 inhibitors in H&N malignancies.
- Evaluate the expression pattern of GSK-3beta in human H&N benign tissue and malignant tumors.

METHODS 1

Commercial TMA, obtained from BioChain, Newark, CA (cat. Z7020051), contained 48 surgical resection cases of inflammatory, benign and malignant tumor tissues of the neck, oro- and naso-pharynx, larynx and salivary glands. They were fixed in 10% neutral buffered formalin for 24 hours and processed using identical standard operating procedures (SOPs).

METHODS 2

Immunohistochemical staining:
- Performed on paraffin section of the TMA.
- The paraffin section of TMA was deparaffinized, and antigen retrieval was carried out in citric buffer in microwave for 10 min.
- The section was incubated in 1% hydrogen peroxide for 10 minutes to quench endogenous tissue peroxidase and then incubated with the anti-GSK-3β antibody, Zapico M, et al., Glycogen synthase kinase-beta (GSK-3β) participates in nuclear β3 activity leads to epigenetic alterations of cancer.  Cancer Letters (2016), doi: 10.1016/j.canlet.2016.07.006.

Figure 1: (A) GSK-3β expression in salivary gland, core H4, H&N TMA (NEG). (B) Nuclear and cytoplasmic GSK-3β expression (+), squamous cell carcinoma, pharynx, core E5, H&N TMA; (C) Nuclear and cytoplasmic GSK-3β expression (+), squamous cell carcinoma, oral cavity, core E9, H&N TMA

Figure 2: Tissue Array Diagram. Tissue Type and GSK-3β expression GSK-3β nuclear expression was defined as positive staining of more than 50% of cancer cell nuclei throughout the tumor regardless of cytoplasmic staining.

RESULTS

Table 1: Frequency of GSK-3β expression in malignant and benign head and neck TMA specimens.

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Number of specimens</th>
<th>GSK-3β expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCHN</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>NPC</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>H&amp;N malignancies other than SCC</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Benign H&amp;N (salivary)</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Benign Salivary gland tissue</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Malignant Salivary gland tissue</td>
<td>22</td>
<td>22</td>
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</table>

CONCLUSIONS

Our results demonstrate that there is aberrant nuclear expression of GSK-3β in SCCHN. This finding supports the clinical exploration of GSK-3β inhibitors in SCCHN and further study of GSK-3β as a potential prognostic and predictive biomarker for risk of recurrent disease and chemoresistance in patients with advanced SCCHN. The role of aberrant nuclear expression of GSK-3β in salivary gland malignancies merits further study.

REFERENCES