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

Maria Matsangou[1,3] Andrey Ugolkov[1,2,5] Tim J. Taxter [1,4] Andrew P. Mazar [1,2,5] and Francis J Giles[1,3]

[1]Center for Developmental Therapeutics, [2]Department of Pharmacology, [3]Department of Medicine Division of Hematology-Oncology, [4]Department of Pathology, Feinberg School of Medicine, and [5]Chemistry of Life Processes Institute, Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL

Northwestern Medicine® Developmental Therapeutics Institute

Robert H. Lurie Comprehensive Cancer Center of Northwestern University

Northwestern University Feinberg School of Medicine

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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 chemo- or radio-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.
- Evaluated 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).

RESULTS

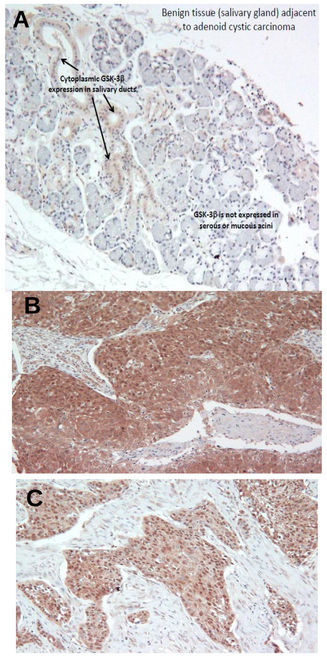


Figure 1: (**A**) GSK-3β expression in salivary gland, core H4, H&N TMA (NEG); (**B**) Nuclear and cytoplasmic GSK-3β expression (+), squamous cell carcinoma, pharynx, core E6, H&N TMA; (**C**) Nuclear and cytoplasmic GSK-3β expression (+), squamous cell carcinoma, oral cavity, core E9, H&N TMA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Benign, inflammation	Salivary gland, inflammation	Polyp	Polyp	Polyp	Polyp	Hemangioma	Hemangioma	Neurofibroma	Neurofibroma	Schwannoma	Schwannoma
B	Neg	Cyt	Neg	Cyt	Cyt	Cyt	Neg	Cyt	Cyt	Cyt	Cyt	Cyt
C	Pleomorphic adenoma	Pleomorphic adenoma	Pleomorphic adenoma	Pleomorphic adenoma	Pleomorphic adenoma	Pleomorphic adenoma	Pleomorphic adenoma	Pleomorphic adenoma	SCC	SCC	SCC	SCC
D	Cyt	Nuc	Nuc	Cyt	Cyt	Nuc	Nuc	Cyt	Nuc	Cyt	Nuc	Nuc
E	SCC	SCC	SCC	SCC	SCC	SCC	SCC	SCC	SCC	Nasopharyngeal carcinoma	SCC	Mucoepidermoid carcinoma
F	Nuc	Neg	Nuc	Nuc	NA	Nuc	Cyt	Nuc	Nuc	Cyt	Nuc	Cyt
G	ACC	ACC	ACC	ACC	ACC	Rhabdomyosarcoma	Chondrosarcoma	Non-Hodgkin B cell lymphoma	Diffuse large B-cell lymphoma	Metastatic adenocarcinoma	Metastatic nasopharyngeal carcinoma	Metastatic SCC
H	Nuc	Nuc	Nuc	Neg	Cyt	Neg	Nuc	Nuc	Cyt	Cyt	Cyt	Nuc

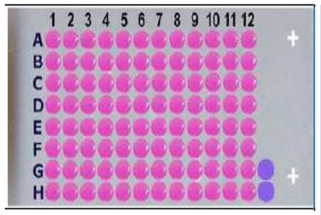
Figure 2: **Tissue Array Diagram**: Tissue Type and GSK-3β expression
GSK-3β nuclear expression was defined as positive staining of more than 50% of cancer cell nuclei throughout the tumor regardless of cytoplasmic staining.

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

	SCCHN	NPC	H&N malignancies other than SCC	Benign H&N (non-salivary)	Benign Salivary gland tissue	Malignant Salivary gland
Number of specimens N=48	15	2	5	11	9	6 (5ACC,1MEC)
GSK-3β Nuclear POS (N)	11	2	2	0	4	3 (ACC)
GSK-3β Nuclear POS (%)	All H&N malignancies: 68% SCC: 73%			0%	44%	60% ACC

SCCHN= Squamous cell carcinoma of Head and Neck, NPC=Nasopharyngeal carcinoma, ACC= Adenoid cystic carcinoma, MEC= mucoepidermoid carcinoma

METHODS 2



The TMA contained 96 cores: 48 total cases (2 tissue cores/case), e.g. cores A1, B1 represent samples from same case, taken from different parts of the specimen.
•22 malignant H&N samples (15 SCCHN, 2 nasopharyngeal and 5 other histology)
•11 Benign H&N samples
•9 Benign Salivary gland samples
•6 Malignant salivary gland samples

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 peroxidase for 10 minutes to quench endogenous tissue peroxidase and then incubated with the anti-GSK-3β antibody (Cell Signaling, Danvers, MA) overnight at +4C. The slide was stained using a standard EnVision+ System-HRP kit (DAKO, Carpinteria, CA) according to the manufacture's protocol.
- Immunohistochemical reactions were developed with diamino-benzidine as the chromogenic peroxidase substrate, and slide was counterstained with hematoxylin.
-**We defined GSK-3β nuclear accumulation as positive staining of >50% of cancer cell nuclei throughout the tumor regardless of cytoplasmic staining.**

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 chemo- or radio-resistance in patients with advanced SCCHN. The role of aberrant nuclear expression of GSK-3β in salivary gland malignancies merits further study.

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