Beta spectrin staining is associated with higher risk of recurrent disease in oral tongue squamous cell carcinoma

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Introduction

Biomarkers are used to guide treatment for multiple different types of cancers, including breast, melanoma, and lung, and to help personalize management and optimize cancer treatment. However, biomarker discovery for head and neck cancer is relatively new and limited, and no biomarkers have been identified that are associated with treatment responsiveness in HPV-negative squamous cell carcinoma (SCCa). Thus, a means to predict survival in these patients is lacking[1, 2]. Currently, treatment plans and estimates of prognosis for patients presenting with oral tongue squamous cell carcinoma (OTSCC) are determined based on stage. Previous work by our group evaluating candidate biomarker genes for HPV-negative SCCa of the oral cavity found that expression of the *SPTBN* gene was associated with significantly higher risk of mortality as compared to oral cancer patients that did not express *SPTBN.* The current work was designed to determine if these results found in gene expression data would correlate with immunohistochemistry analysis. We sought to examine the relationship between the expression of the protein product for which *SPTBN* codes, the protein spectrin beta chain non-erythrocytes-1, and several clinical outcomes.

Methods

After IRB approval, patients treated at our facility who underwent surgery for OTSCC from 2010-2018 were identified through our Cancer Registry. Tissue blocks were retrieved and three sections of each tissue block were fixed to glass slides and antigen retrieval was performed. One section was stained with anti-spectrin mouse Ab (Invitrogen MA3-062), another was stained with anti-spectrin rabbit Ab (abcam ab72239), and finally, a blocking buffer, which served as a control, was added to the last section. After incubating the tissues with appropriate secondary antibodies, followed by diaminobenzadine incubation and hematoxylin counterstain, the slides were examined, and the anti-spectrin mouse Ab was found to have stronger binding. Thus, the sections washed with anti-spectrin mouse Ab were used for further pathological analyses. Stained tissues were scored by a specialized head and neck pathologist, who was blinded to characteristics of the tumors such as staging, recurrence, and death, all of which were known to the researchers. Notably, the tumor cells themselves stained only weakly for spectrin, but the adjacent muscle cells that were being invaded by the tumor cells stained more strongly. Spectrin tissue score was therefore based on the level of spectrin protein expression in the muscle cells. Tumors were also graded for presence of perineural invasion (PNI), worst pattern of invasion (WPOI) from 1-5 and lymphoid response from 1-3.

Results

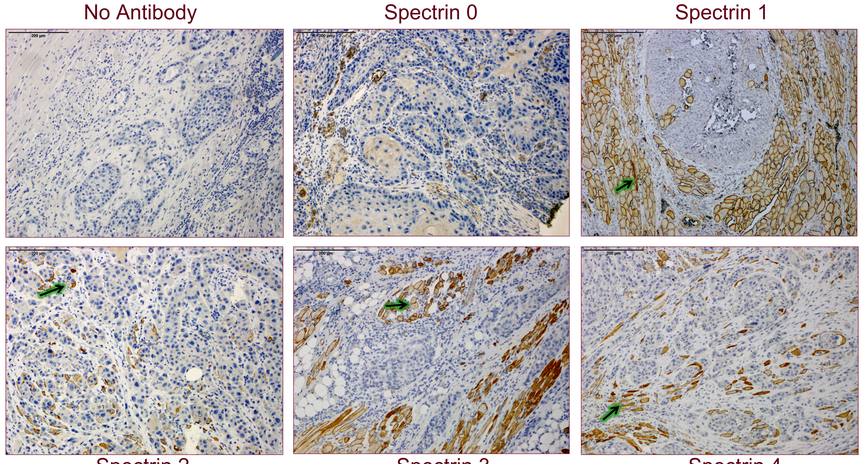

Figure 1: Tumor sections stained and scored for presence of beta spectrin protein. Score 0 indicates absence of beta spectrin, score 4 indicates strongest presence of beta spectrin. Black and green arrows indicate areas of tumor invading spectrin positive muscle, the latter of which is stained brown.

Table 1: Disease Free Survival, n=143

	Unadjusted		Adjusted	
	Hazard Ratio (95% CI)	***p***	**Hazard Ratio (95% CI)**	***p***
Age (per 5-year increase)	1.06 (0.98 – 1.24)	0.11		
Sex (Male vs Female)	1.14 (0.60 – 2.17)	0.68		
Spectrin (per level increase)	1.10 (1.05 – 1.16)	<0.001	1.11 (1.05 – 1.17)	<0.001
Perineural Invasion: Present vs Absent	2.93 (1.50 – 5.72)	0.002	2.34 (1.08 – 5.09)	0.03
Worst Pattern of Invasion (per level increase)	1.49 (1.07 – 2.07)	0.02		
Lymphoid Response (per level increase)	1.64 (1.05 – 2.55)	0.03	1.59 (0.98 – 2.58)	0.06

Results (cont'd)

OVERALL SURVIVAL: Controlling for age, PNI status, and stage, ***the risk of all-cause mortality was nominally higher for patients with elevated spectrin levels (HR = 1.06, 95% CI: 0.99 – 1.14; p=0.08)***. Conversely, controlling for all other variables in the model, the rate of mortality was 2.68 (95% CI: 1.13 – 6.35) times higher for patients with PNI, and every 5-year increase in age increased the rate of mortality by approximately 34% (HR = 1.34, 95% CI: 1.10 – 1.64; p=0.004).
DISEASE FREE SURVIVAL: Controlling for other variables, ***the rate of recurrence or disease-related mortality increased by approximately 11% for every one-level increase in spectrin (HR = 1.11, 95% CI: 1.05 – 1.17; p=0.001)***. Similarly, the rate of recurrence or disease-related mortality was 2.34 (95% CI: 1.08 – 5.09) times higher for patients with PNI (p = 0.03).
ODDS OF POSITIVE N STAGE: There was no association between patients' spectrin level, PNI or lymphoid response and N-stage status. Conversely, controlling for all other variables in the model, each level increase in patients' WPOI increased the odds of a positive N-stage status by approximately 84% (OR = 1.84, 95% CI: 1.23 – 2.77; p=0.003).

Discussion

As discussed in the Methods section, the spectrin positive cells were not the tumor cells themselves, but skeletal muscle cells intermixed with/being invaded by tumor. We hypothesize that the association we found in this study between spectrin staining and disease free survival is a reflection of an increasingly invasive tumor subtype that is more likely to recur after treatment. Future work will be aimed at determining biomarker panels that may combine factors such as spectrin staining, PNI and age, to guide treatment decisions in patients with OTSCC.

Conclusion

Spectrin staining of OTSCC-associated muscle cells is associated with a decrease in disease free survival. This may reflect an additional important risk factor for local recurrence in this patient population that can guide the need for adjuvant therapy.

References

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