NEURAL STEM CELLS IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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ABSTRACT

The initial intent of this study was to examine the origins of Primary Central Nervous System Lymphoma (PCNSL), a neoplasm whose oncogenesis in immunocompetent patients is incompletely understood. With growing information regarding the remarkable plasticity of neural stem cells, and establishment of relationships between hematopoietic and neural stem cells, we hypothesized that PCNSL arises from neural stem/progenitor cells rather than lymphocyte precursors from elsewhere in the body. Markers of neural stem cells were chosen for immunohistochemical (IHC) staining of 5 PCNSL cases and all cases contained Sox2 positive cells, whereas 8 of 9 non-CNS B cell lymphomas so stained had no positive cells. Double-staining with Sox2 and CD20, a B-cell marker, showed no co-localization of Sox2 and CD20, and no CD20-positive tumor cells had Sox2 immunopositivity. Staining of 5 metastatic carcinomas and 1 metastatic melanoma revealed a similar pattern of immunopositivity both regarding sox2 expression and subsequent double-staining with low molecular weight cytokeratins. These findings suggest that neural stem cells are enriched in PCNSL as a reaction rather than as a source of the tumors and that sox2 expression is indicative of a neural progenitor/stem cell response to non-neural neoplasms. It follows that not all stem cell marker-positive cells in a tumor are tumor stem cells. Further understanding of the reactive response of neural stem cells is needed for understanding of neural neoplasm pathology. The presence of these findings in diverse central nervous system neoplasms and manipulation of the observation for therapeutic benefit have yet to be explored.

INTRODUCTION

- Hypotheses of PCNSL oncogenesis have focused on seeding, recruitment, and homing of malignant lymphocytes to the CNS from the periphery[1,2].
- Studies have shown the potential for neural stem cells to differentiate into hematopoietic cells[3], so we hypothesized that PCNSL may arise from a neural stem/progenitor cell.
- IHC staining of 5 PCNSL cases was performed with 6 neural stem/progenitor cell markers.
- Only one marker, sox2, showed significant results.
- Sox2 is an HMG box transcription factor and one of the Yamanaka factors which maintain stem cell pluripotency.
- Sox2 positive cells exist in various cancers, but it has primarily been studied as a marker for neural stem cells.
- Double-staining was performed with sox2 and CD20, a marker for B-cells to examine the true nature of the cells.
- Co-localization of sox2 and CD20 would suggest the sox2 positive cells are stem cells of the tumor, while lack thereof would suggest that the sox2 positive cells are reactive neural stem cells and not tumor cells.
- Double-staining was repeated with sox2 and CAM5.2 in 5 metastatic carcinomas to brain, and with sox2 and HMB45 in 1 metastatic melanoma to brain.

MATERIALS AND METHODS

***Tissue Preparation*.**

The tissues examined were surgically removed from 2008 to 2012 and pulled from the files of the Department of Pathology of the University of Missouri Hospitals and Clinics.

***Initial Immunohistochemistry*.**

Immunohistochemistry using the antibodies sox2, B-tubulin, GFAP, CD133, synaptophysin, and nestin was performed on 5 cases of CNS lymphoma, 5 cases of glioblastoma, and 9 systemic lymphomas.

Secondary Immunohistochemisty.

Sox2/CD20 double-staining, sox2/CAM5.2, and sox2/HMB45 was performed on 5 PCNSL cases, 5 metastatic carcinomas to brain and 1 metastatic melanoma to brain, respectively.

RESULTS

Case	Sox2	CD133	Nestin	B-tubulin	GFAP	Synaptophysin
Glioma 1	+	-	-	+	+	+
Glioma 2	+	+	+	+	+	+
Glioma 3	+	-	+	+	+	+
Glioma 4	+	+	+	X	+	+
Glioma 5	+	+	+	+	+	+
PCNSL 1	+	-	-	-	-	+*
PCNSL 2	+	-	-	-	-	+*
PCNSL 3	+	-	-	-	-	-
PCNSL 4	+	-	-	-	-	+*
PCNSL 5	+	+	+	-	-	+*
Syst Lymph 1	-	-	-	-	-	-
Syst Lymph 2	-	-	-	-	-	-
Syst Lymph 3	-	-	-	-	-	-
Syst Lymph 4	-	-	-	-	-	-
Syst Lymph 5	-	N/A	N/A	N/A	N/A	N/A
Syst Lymph 6	+	N/A	N/A	N/A	N/A	N/A
Syst Lymph 7	-	N/A	N/A	N/A	N/A	N/A
Syst Lymph 8	-	N/A	N/A	N/A	N/A	N/A
Syst Lymph 9	-	N/A	N/A	N/A	N/A	N/A

Syst Lymph: Systemic Lymphoma, *: Pattern of immunopositivity was consistent with trapped neurons rather than tumor cells, X: No viable tissue remaining, N/A: Was not performed.

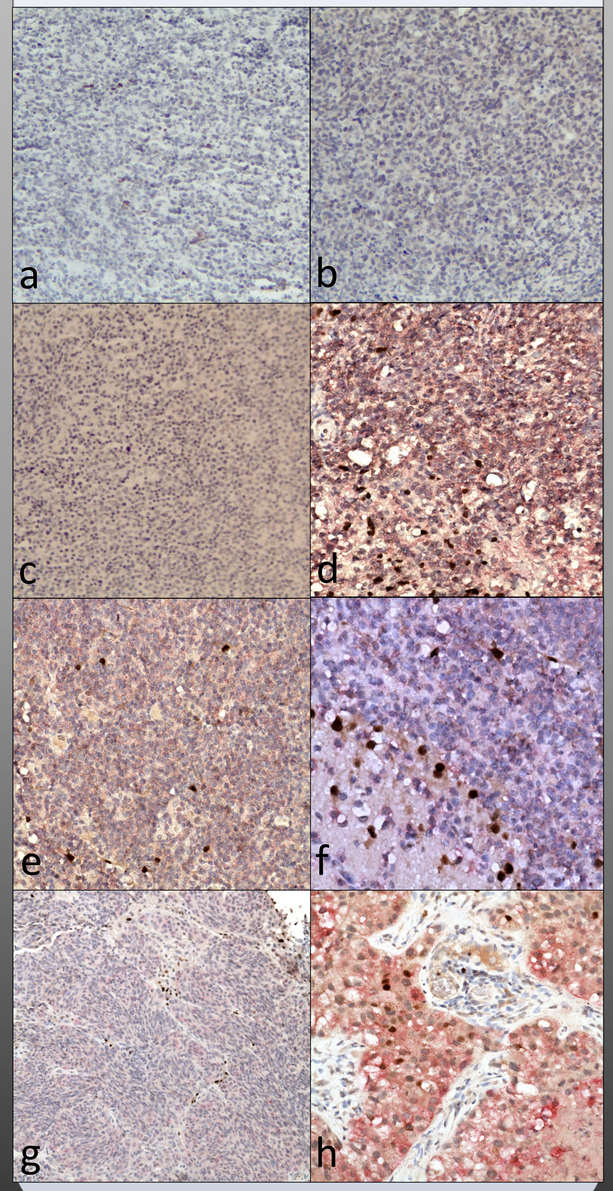
Case	Sox2	CAM5.2	HMB45	Co-localization
Metastatic Carcinoma 1	+	+	N/A	-
Metastatic Carcinoma 2	+	+	N/A	-
Metastatic Carcinoma 3	+	+	N/A	-
Metastatic Carcinoma 4	+	+	N/A	-
Metastatic Carcinoma 5	+	+	N/A	-
Metastatic Melanoma 1	+	N/A	+	-

RESULTS SUMMARY

- All 5 PCNSL cases were positive for sox2.
- 8 of 9 systemic lymphomas were sox2 negative.
- No other neural stem cell marker showed significant immunopositivity in the PCNSL cases.
- Sox2/CD20 double-stain showed no co-localization.
- Sox2/CAM5.2 double-stain showed no co-localization.
- Sox2/HMB45 double-stain showed no co-localization.

IMMUNOSTAINS

a) Positive sox2 immunostain in PCNSL.
b) Negative sox2 immunostain in systemic lymphoma.
c) Negative CD133 immunostain in PCNSL.
d) Double-stain of sox2 (brown) and CD20 (red) in PCNSL No co-localization is seen. Sox2 positivity at tumor margin.
e) Negative co-localization of sox2 (brown) and CD20 (red) in PCNSL.
f) Same PCNSL case as in image e, but demonstrating sox2 positive cells at the tumor margin.
g) Negative co-localization of sox2 (brown) and CAM5.2 (red) in metastatic carcinoma to the brain.
h) Negative co-localization of sox2 (brown) and CAM5.2 (red) in metastatic carcinoma to the brain at higher magnification.



DISCUSSION

Only one of six markers of neural stem/progenitor cells showed significant immunopositivity in PCNSL which does not help to elucidate the cell origin of this rare tumor.

As none of the tumors showed co-localization of sox2 with the secondary cell marker, sox2 positive cells are likely reactive neural stem cells behaving in response to neural injury rather than primary tumor stem cells involved in tumor oncogenesis. Also, because sox2 positivity was negative in 8 of 9 systemic lymphomas, sox2 induction is likely a brain-derived process.

Consistent with this finding, sox2 immunopositivity followed an infiltrative pattern of sox2 positive cells primarily existing at tumor margins rather than in the bulk of the tumor, demonstrated in images d and f. There is not considerable literature on sox2 positivity in reactive cells to CNS neoplasia, however its presence near other CNS injury sites has been noted in a few instances.

Sox2 positive neural stem/progenitor cells have been noted around areas of injury in chronic demyelinating disease[4] and near the injury of hypoglossal nerve avulsion[5]. Also, a “modest” increase in sox2 positive cells around the injury site of mice undergoing stab wounds has been noted, although no increase in proliferation or migration pattern was observed[6].

Further study into sox2 immunopositivity and role in reactive neural stem cell function is needed, both with neural and non-neural neoplasms and other forms of CNS injury.

CONCLUSIONS

- Sox2 is indicative of a neural response to non-neural neoplasms.
- Not all stem cell markers mark tumor stem cells.
- Consideration and study must be given to a better understanding of reactive neural stem cell physiology, identification, and potential therapeutic use.

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