CNTF Receptor Subunit-alpha Is a Marker for Glioma Cancer Stem Cells and Correlates with Tumor Grade in Primary Brain Tumors

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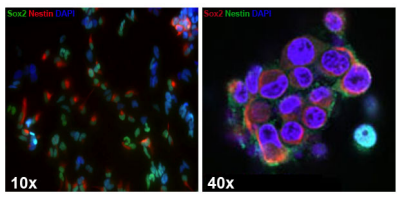
Introduction

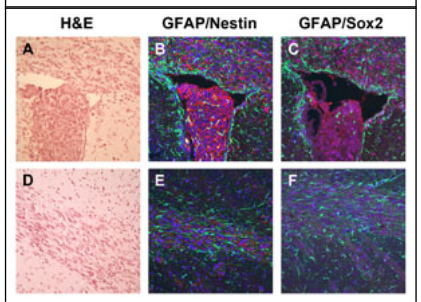
In vivo experiments in glioma have shown that cancer stem cells (CSCs) are uniquely resilient to current treatment regimens, contributing to both cancer resistance and recurrence. However, due to the lack of specific markers, identification of CSCs has presented a major challenge to their targeting and achieving cure. To identify new biomarkers and potential therapeutic targets we sought to find unique proteins from brain tumor-derived CSCs.

Methods

We compared the proteomes of glioma-derived stem cells to their differentiated progeny to identify novel CSC-specific proteins. CSCs were confirmed using stem cell neurosphere morphology and markers as well as a xenograft model showing the tumorigenic characteristics of implanted CSCs in brain parenchyma. Proteomic analysis was performed using a novel "multidimensional" capillary isoelectric focusing nano-reversed -phase liquid chromatography with tandem mass spec (CIEF-nRPLC-MS). The expression of potential CSC marker CNTFRa was confirmed through western blot and immunohistochemistry. We also assessed its potential for clinical application.



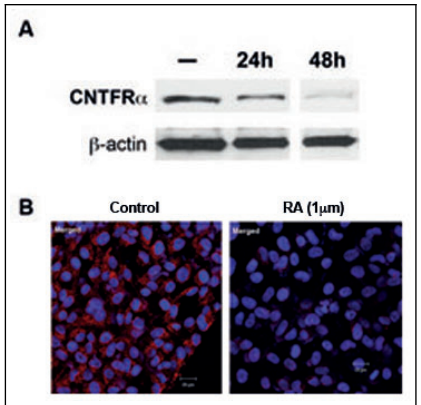
IF staining for CSC markers Sox2 and Nestin



Xenograft CSC mouse model showing infiltrative growth and stem cell marker expression of implanted CSCs in brain parenchyma.

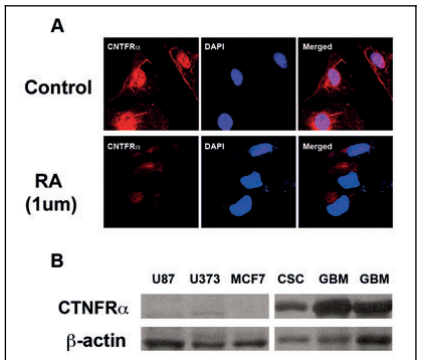
Results

Of several proteins found to be differentially expressed, we focused our study on ciliary neurotrophic factor receptor subunit-alpha (CNTFRa) because of its biological relevance in glial cell differentiation. Using western blot and immunofluorescence (IF), we confirmed CNTFRa down-regulation in differentiated CSC progeny and identified CNTFRa expression in patient glioma samples. Additionally, CNTFRa expression rose proportionally in tumors of increasing grade, which highlights its potential use for tumor prognosis.



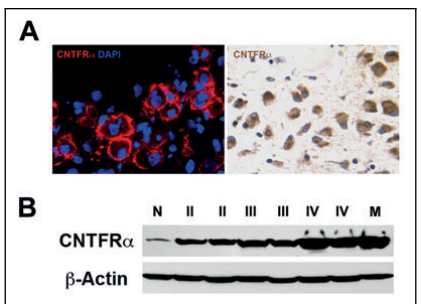
Western blot (A) and IF (B) showing selective expression of CNTFR in undifferentiated CSCs and loss of CNTFR expression in RA treated cells.

The CNTF pathway's role in astroglial differentiation led us to consider CNTFRa genetic mutations as a potential explanation of aberrant CNTFRa expression in undifferentiated CSCs.

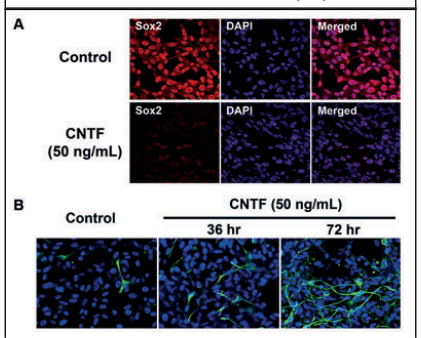


IF and western blot showing selective expression of CNTFRa in medulloblastoma cell line DAOY (A) and GBM cells (B).

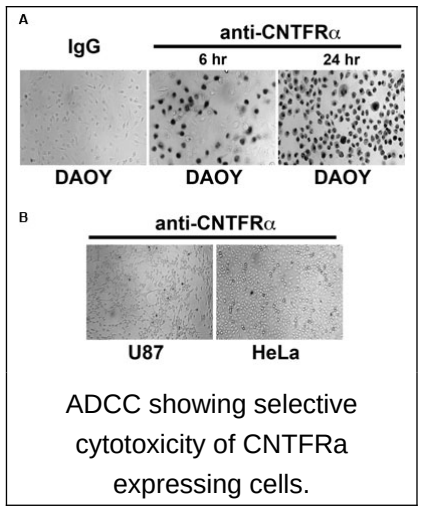
Genetic analysis identified a mutation in only 1 of 32 (3%) gliomas tested, which was insignificant. Further, we found that CNTF ligand was able to drive the differentiation of glioma CSCs and DAOY cells, indicating a properly functioning CNTF receptor in both cell types.



(A) IF and IHC of patient GBM tissue showing CNTFRa expression. (B) Western blot showing increasing CNTFRa expression with increasing glioma grade (I - IV) and medulloblastoma (M).



IF of CSCs treated with CNTF ligand showing Sox2 loss (A) and GFAP gain (B) suggesting differentiation of CSCs and a functional CNTF pathway.



ADCC showing selective cytotoxicity of CNTFRa expressing cells.

Thus, it appears that a genetic mutation in CNTFRa is unlikely to contribute to malignant glioma tumorigenesis.

To assess the potential of CNTFRa as a therapeutic target, antibody-dependent cell-mediated-cytotoxicity (ADCC) was tested and the result showed increased cytotoxicity in CNTFRa-expressing tumor cells.

Conclusions

Our results suggest a critical role for CNTFRa in primary brain tumors, based on robust expression levels in both whole tumor extracts and purified CSCs. CNTFRa may serve as a tool in the histologic diagnosis of glioma and has the potential to be used for targeted imaging. Furthermore, CNTFRa may be a promising therapeutic target, both through receptor-directed immunotherapy and receptor-mediated pro-differentiation effects.