Glioma Stem Cells-targeting peptide isolated through phage display

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Introduction

Glioblastoma (GBM) is the most aggressive brain tumor with high mortality rates and resistance to conventional therapy. Despite the advancement in therapeutic research, the recurrence of GBM appears in 70% of the cases. This has been attributed to the presence of cancer stem cells (CSCs), a small fraction of cells resistant to treatment and capable of self-renewal and tumor initiation. Targeting the Glioma Stem Cells (GSCs) will allow to develop effective therapeutic strategies.

Method

Phage display is a discovery tool that is able to isolate peptides that target a specific cell type through positive and negative selection steps. Using the 7-amino-acid-length peptide library, negative selection was performed against extracellular-matrix-coated plates and non-stem GBM cells. The remaining phage library was applied to CD133+ GSCs, and 4 rounds of positive selection were performed. In parallel, GBM cells were implanted into the brain of NSG mice. The 7-amino-acid-length peptide library was intravenously injected in these mice and 24h later, the tumors were harvested and peptide isolated.

By combining in vivo and in vitro phage display, we obtained one common peptide that could detect GSCs: EYA1.

EYA1 peptide selected as a Glioma stem cells (GSC)-targeting peptide

Figure 1: Glioma stem cells (GSC) targeting peptide isolated through phage display biopanning.

One common peptide was sorted after in vitro and in vivo biopanning designated EYA1 peptide.

EYA1 peptide binds to GSCs

The conjugated EYA1 peptide with Cyanine 5.5 binds more specifically to GSCs than a non-targeting peptide (NT peptide, GGGSGGG-Cy5.5).

Figure 2: EYA1 peptide binds to GSCs in vitro.

A Representative images obtained by Imagestream flow cytometer of stained GSCs. B- Analysis of the Median Fluorescence Intensity of GSCs stained by EYA1 (red) or NT (green) peptides. ****: P<0.0001

EYA1 peptide targets intracranial glioblastoma xenografts

Figure 3: In vivo binding of EYA1 peptide in intracranial glioblastoma xenograft.

Imaging of glioblastoma bearing mice injected intravenously with EYA1 peptide.

A Intravenous injection of EYA1 of NT peptides followed by IVIS imaging in mice after intracranial injection of GBM cells. B Imaging of EYA1 peptide in mice without (left) or with (right) GBM. C-Measure of the Fluorescence Intensity in muscle area, in brain area in mice without GBM (NB, Normal Brain) and in brain area of GBM bearing mice. D-Ex vivo imaging of a normal brain or a brain with a GBM after EYA1 injection in the mice. E-Measure of the Fluorescence Intensity in Non PDX brain or in Intracranial PDX brain. F-Measure of the Fluorescence Intensity in Intracranial PDX brain area without tumor or in the GBM area.

CDH2 as a binding partner for EYA1 peptide

Figure 4: Identification of EYA1 peptide binding partner.

A ELISA assay verified the binding capacity of EYA1 to CDH2 recombinant protein. B-Rembrandt Expression data set showing a better survival of patients with lower CDH2 and D- a higher expression of CDH2 in GBM compared to non tumor tissue. ****: P<0.0001. E-RT-qPCR of CDH2 in non-malignant cells and 3 patient-derived GSCs. ****: P<0.0001

Figure 5: In vitro confirmation of CDH2 as EYA1 binding partner.

E-Representative images and quantitative analysis (F) obtained by Imagestream of GSCs stained with EYA1-Cy5.5 peptide only or after incubation with CDH2 antibody. G-Representative images and quantitative analysis of the MFI (H) obtained by Imagestream of HEK293 cells, GFP transduced 293 cells (GFP cells) or CDH2 transduced cells (CDH2) stained by EYA1-Cy5.5 (G, H) or NT-Cy5.5 peptides (IL, J). *: P<0.05; **: P<0.01.

Conclusion

Phage display biopanning can isolate a tumor-targeting peptide specific for GBMs. Through this technic, we isolated a peptide that is binding to GSCs and that has affinity with N-cadherin. Tumor-homing peptides can lead to the development of strategies for the treatment and diagnosis of glioblastomas. For example, the combination of EYA1 peptide with Chimeric Antigen Receptor (CAR)-T could be used to target GBM and GSCs.

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