Immunotherapeutic targeting of NY-ESO-1 in glioblastoma and meningioma with hypomethylating agent, Decitabine

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Background

Glioblastoma and meningioma are the most common primary brain tumor and the most common brain tumor in adults over 35 years of age, respectively[1]. Despite major efforts in developing new, effective therapies and stratifying patients based on the genetic profile, glioblastoma patients succumb to the disease with median survival of 14.5 months due to its high recurrence rate. Of meningioma patients, 20-25% are recurrent and/or histologically aggressive (WHO Grade II and III) with high morbidity and mortality[2,3]. Development of consistently and uniformly expressed immunotherapeutically targetable cancer antigen from glioblastoma and meningioma can lead to effective immunotherapy against these aggressive brain tumors.

Cancer/testis antigen (CTA) genes are normally suppressed in somatic cells after embryonic development but can be reactivated in certain malignancies[4]. Therefore, CTAs such as NY-ESO-1 (Cancer/Tests Antigen 1B, *CTAG1B*) is an attractive antigen for targeted immunotherapy.

Project Goals

1. Test efficacy of DNA methyltransferase inhibitor (DNMTi) named decitabine (DAC) in inducing the expression of NY-ESO-1 in primary glioblastoma and meningioma cell lines
2. Identify sub-population responsible for NY-ESO-1 induction by single cell RNA sequencing
3. Establish mechanistic explanation for DAC-mediated NY-ESO-1 induction through bi-sulfide sequencing
4. Test whether DAC changes methylation profile subtypes of glioblastoma and meningioma (for example, from cold tumor to hot tumor)
5. Test survival benefit of DAC treatment in xenograft NSG mouse model with NY-ESO-1 targeting T cell therapy

Results

Figure 1

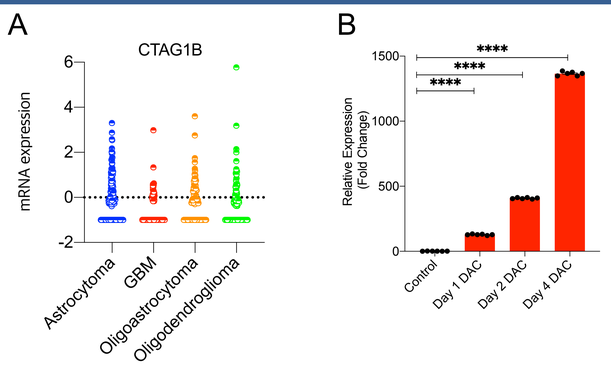


Figure 1- NY-ESO-1 transcription is low in brain tumors and can be induced by DAC treatment. *CTAG1B* expression level is downregulated in most of brain tumors including astrocytoma, GBM, oligoastrocytoma, and oligodendroglioma (Fig. 1A). When immortalized glioblastoma cell line, U251, is treated with 1 μM decitabine (DAC), *CTAG1B* expression is induced in a timepoint dependent manner.

Figure 2

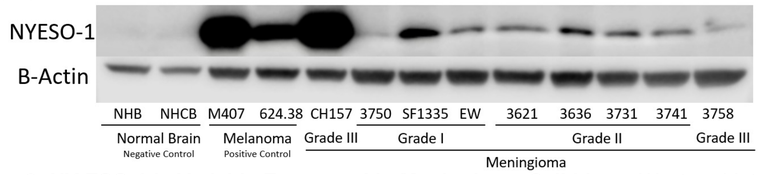


Figure 2- NY-ESO-1 is Variably Expressed in Meningioma Cell Lines. Western blot of various primary and established meningioma cultures, with normal brain as negative and melanoma as positive control. NHB:normal human brain. NHCB: normal human cerebellum.

Figure 3

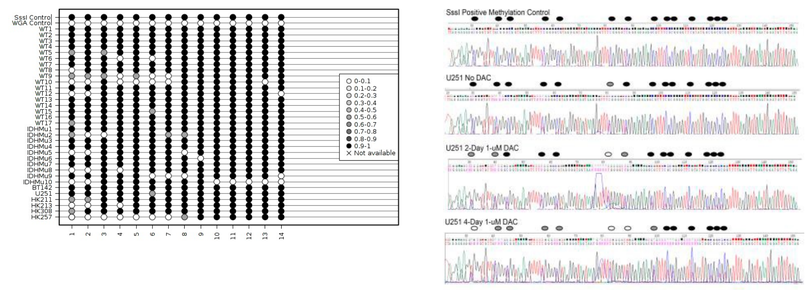


Figure 3- CpG island of NY-ESO-1 gene is methylated in the majority of GBM and can be hypomethylated by DAC treatment. CpG island analyzed contains 14 CpG sites, black circles show patients where CpG sites completely methylated, grey partially demethylated (C/T peak ratio 0.4-0.6), white completely demethylated. First row shows CpG methyltransferase positive control (SssI). Second row shows whole genome amplification negative control (WGA). Subsequent rows show Isocitrate Dehydrogenase (IDH) wildtype patient tumors (WT) and IDH mutant patient tumors (IDHMu) (Fig. 3A). Partial demethylation by DAC treatment increases NY-ESO-1 expression. U251 cells treated with 1 uMDAC for 2 or 4 days, and then left to incubate in clean medium until 7-day timepoint. (Fig. 3B)

Figure 4

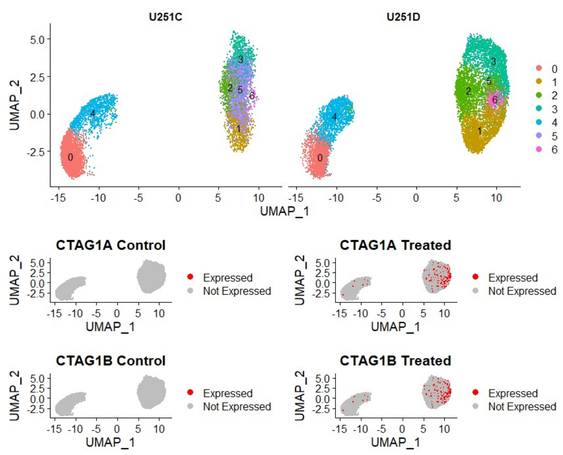


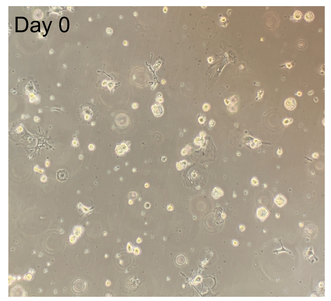
Figure 4- Single Cell RNA Sequencing reveals that DAC Treatment Expands and Diminishes Some Clusters of U251 Cells and CTAs such as NY-ESO-1 are Induced in a Sub-population of Cells Spanning over Multiple Clusters. UMAP projection of CTAG1B which encodes NY-ESO-1. UMAP projection of other CTA MAGEA4 and CTAG2. Similar expression localization in UMAP projection, indicating coordinate upregulation.

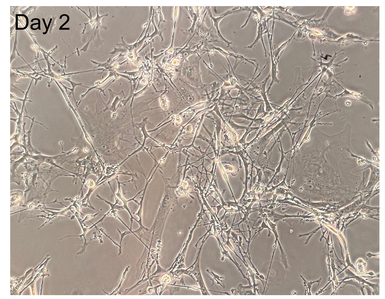
Conclusions

- NY-ESO-1 is downregulated in multiple brain tumor types
- NY-ESO-1 can be induced by decitabine treatment in a timepoint dependent manner up to >1000x
- Primary meningioma cell lines show variable expression levels of NY-ESO-1 protein level
- CpG islands for NY-ESO-1 show hypermethylation, which may explain the low expressions in various brain tumors
- Decitabine treatment hypomethylates the CpG island for NY-ESO-1 gene
- Single cell RNA sequencing reveals shifts in some cluster sizes and induction of CTAs such as NY-ESO-1 expression in a subset of tumor cell population spanning over various clusters of cells

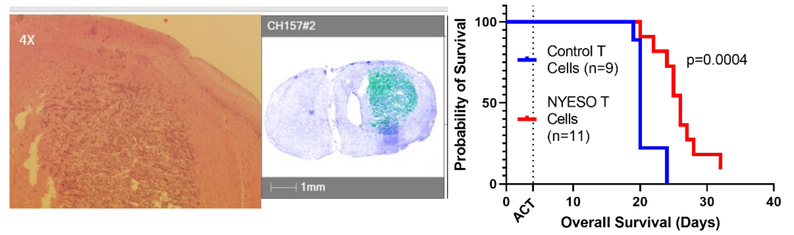
Future Directions

- **Look into more global changes in the methylation profile upon DAC treatment in immortalized and primary cell lines by using Infinium MethylationEPIC Array that allows you to look at interrogate over 850,000 methylation sites quantitatively across the genome at single-nucleotide resolution**
  - **Question #1**:Would there be a shift in methylation landscape to result in changes in tumor subtype[5]?
  - **Question #2**:If there is a subtype change, would it be towards mesenchymal subtype (in case of GBM) to make the tumor more susceptible to immunotherapy such as DC-vaccine?
  - **Question #3**:What other pathways or CTAs can be taken advantage of in order to improve efficacies of the current immunotherapies?





- **Can DAC treatment result in survival benefit in an orthotopic xenograft mouse model injected with various primary meningioma cell lines and treated with engineered NY-ESO-1 specific, syngeneic T cell therapy?**



References

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