Immortalized HMy2/Tumor Hybridoma Induce Different Antigen-Specific CTL Clones *In-Vitro*

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Background

- Hepatocellular carcinoma (HCC) is the 5th most common cancer and the 2nd and 6th leading cause of cancer-related death in the less and the well developed countries, respectively, worldwide[1].
- An estimate of global 783,000 new cases and 746,000 deaths occurred in 2012.[1]
- New chemotherapeutic agents (e.g. Sorafenib) were proved to induce better survival; however, tumor relapse and patient un-eligibility are still the most barriers[2].

Figure 1: Normal and diseased liver

- Immunotherapy represents an attractive alternative approach of high specificity and sensitivity, long lasting and better safety.
- Induction of HCC antigen-specific cytotoxic T lymphocytes (CTL) for adoptive therapy has shown great interests in recent studies in order to enhance quality and productivity of the activated T cell clones.
- We generated an in vitro model for effortless and costless induction of Glypican-3 (GPC-3) and α-fetoprotein (AFP) antigen-specific CTL clones (as these TAAs are expressed in around 80% of HCC cases)[3]

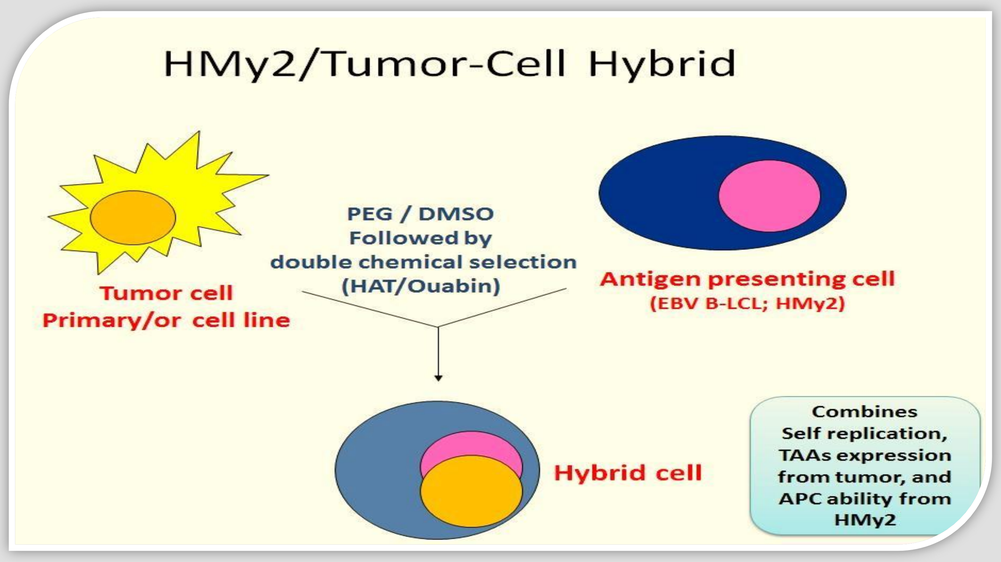

Figure 2: Summary of hybrid cells production and growth selection

Aims

We aimed to investigate the ability of a hybrid model (HepG2xHMy2), generated by fusion of HCC cell line (HepG2) and EBV-sensitized B-lymphoblastoid cell line (as a professional APC), to induce allogeneic GPC-3 and AFP-specific and functional cytotoxic T cell clones in vitro, for adoptive cytotoxic T cell immunotherapy of HCC.

Methods

1- Hybrid cells generation and clonal selection: Figure 2 summarizes the processes of fusion, physical and chemical selection of the fusion product.

2- Phenotypic characterization of the fusion product: Co-stimulatory CD markers and HLA expression by FACS analysis

3- GPC-3 and AFP expression by parent and hybrid cells using RT-PCR

4- Lymphoproliferative ability of the hybrid cells using MTT colorimetric assay following MLR with allogeneic HLA-A2+ PBMC of healthy donors

5- Long-term co-culture for priming CTL clones in presence of rhIL-2

6- Pentamer/ tetramer staining of HLA-A2-restricted GPC-3 or AFP-peptide-specific CD8+ T cell clones

7- Cytokine release and cellular cytotoxicity assays for the induced CTL clones using IFN-ϒ ELISpot and fluorometric DELFIA® EuTDA Cytotoxicity kit

Results

- ✓ Great stability, immortality and phenotypic homogeneity of the generated hybrid cell line
- ✓ High expression profile of co-stimulatory CD markers and candidate tumor antigen (GPC-3 and AFP, and the house keeping GAPDH), (Fig 3A & 3B)

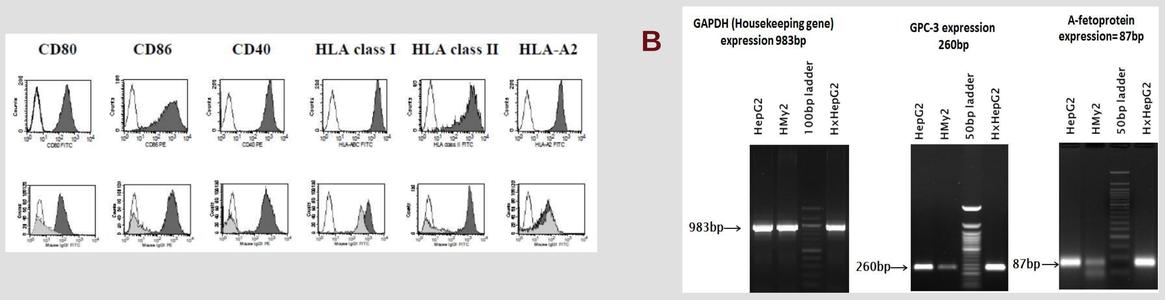

Figure 3: **A: CD marker and HLA expression profile** by HMy2 (top panel) and parental HepG2 with the generated hybrid HepG2xHMy2 (bottom panel). **B; GPC-3, AFP and GAPDH expression** within the hybrid and its parental cell lines as labeled on top of the RT-PCR bands.

- ✓ Great ability to induce proliferative T cell response in allogeneic MLR (Fig. 4A)
- ✓ Long-term co-culture of hybrid cells with allogeneic normal HLA-A2+ PBMC induces T cell clones with the following properties:

1- GPC-3 or AFP, HLA-A2-restricted peptide-specificity as shown by FACS analysis of pentamer or tetramer/CD8 stained T cell cultures (Fig. 5A & 5B)

2- IFN-ϒ –release following sensitization with antigen-expressing cell lines (Fig. 4B)

3- Specific cellular cytotoxic activity against A*02:01-restricted GPC-3 $_{144\text{-}152}$; FVGEFFTDV or hAFP$_{158–166}$; FMNKFIYEI peptide-pulsed T2 cells (Fig. 5C)

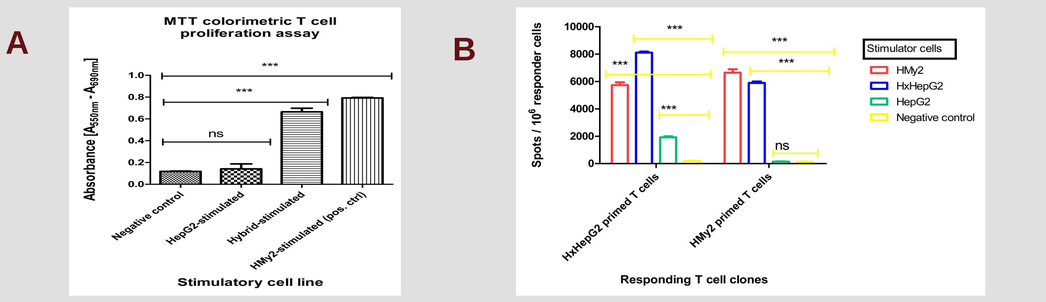

Fig. 4: A; Lymphoproliferative assay using parental or hybrid cell lines in MLR with allogeneic T cells of healthy donors. B; IFN-ϒ release ELISpot assay using activated T cells as effectors and parental or hybrid cell lines as stimulators.

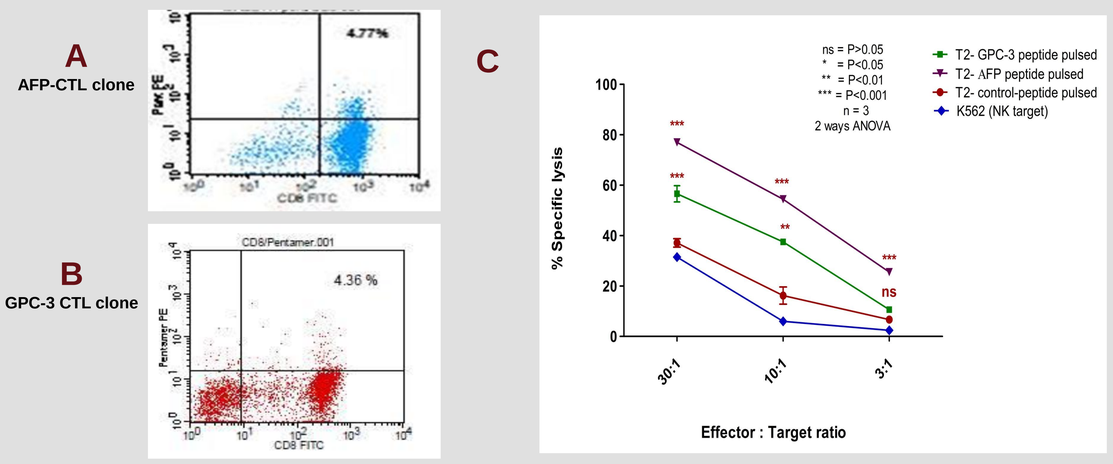

Fig. 5: A & B; Pentamer staining and FACS analysis of AFP and GPC-3 antigen-specific activated CTL clones respectively. B; Cellular cytotoxicity assay using activated T cells as effectors and relevant peptide-pulsed T2 cells as target.

Conclusions

AFP and GPC-3 antigens are widely expressed in HCC but very low or not at all in normal tissues. A number of their epitopes were recognized by CD8+ and CD4+ T cells, a phenomenon which recommended them as target for HCC immunotherapy[4]. Previously, we have shown the feasibility of ex vivo induction of CTL clones specific for TAAs expressed by hematological malignancies using similar hybrid model[5]. In this study, we have proved the ability of induction of AFP and GPC-3 peptide-specific allogeneic CTL clones using HepG2xHMy2 hybrid cell line *ex vivo*. Such T cell clones might be a robust candidate for HCC immunotherapy.

References

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