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. An estimate of global 783,000 new cases and 746,000 deaths occurred in 2012. New chemotherapeutic agents (e.g. Sorafenib) were proved to induce better survival; however, tumor relapse and patient un-eligibility are still the most barriers.

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 allogenic 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 HepG2-A2 restricted peptide specific CD8+ T cell clones
7- Cytokine release and cellular cytotoxicity assays for the induced CTL clones using IFN-Y ELISpot and fluorometric DELFIA® EuTOA Cytotoxicity kit

Background

- 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)

Aims

We aimed to investigate the ability of a hybrid model (HepG2×HMy2), 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.

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)
- 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- INF-Y release following sensitization with antigen-expressing cell lines (Fig. 4B) 3- Specific cellular cytotoxic activity against A*0201-restricted GPC-3 (Fig. 5C) and AFP (Fig. 5D).

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.

References

5. MOHAMED, Y. S., et al., 2012. Long-lived fusions of human haematological tumour cells and B-lymphoblastoid cells induce tumour antigen-specific cytotoxic T-cell responses in vitro. Immunology, 217, 719-729