

# Identification and characterization of human hepatocellular carcinoma-associated antigens

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Hepatocellular carcinoma (HCC) ranks the second to the third highest incidence of cancers and the second highest cause of cancer death in China. HCC is resistant to chemo- and radiotherapy. To survey the possibility using immunotherapy for the elimination of residual cancer cells after surgical resection, we have been searching for the expression of tumor-associated antigens in HCC tissues and the immune response in HCC patients to the tumor antigens. A clue to an immune response in HCC patients is that the tumor-infiltrating cells including CD4 and CD8 T cells are often observed in HCC pathological sections.

The cells of HCC express a variety of tumor-testis (CT) antigens. By RT-PCR, we have found 15 known CT antigens expressed in HCC. Of these, 6 CT antigens (MAGE-A1, -A3, -B1, -B2, SSX1, CTP11) are expressed in over 50% of the HCC specimens tested; another 6 CT antigens (NY-ESO-1, MAGE-A10, MAGE-C1, SSX2, SCP-1, etc.) are expressed in over 30-49% of the HCC specimens. The frequency of one HCC specimen expressing 2 and 3 CT antigens is 70-80% and 50-70%, respectively. It lays the basis for the design of a multivalent tumor antigen vaccine.

For the identification of novel tumor antigens in HCC, we have employed the methods of SEREX, SSH, bioinformatics and proteomics. There are four types of novel tumor antigen-encoding genes that have been cloned and characterized. (i) *Cancer-testis antigen*. It includes five novel CT antigens, HCA 661 (CT30), HCA587 (identical to MAGE-C2), FATE/BJ-HCC-2 (CT43), TPTE/BJ-HCC-5 (CT44) and ZNF165. (ii) *Cancer-placental antigen*. A CP1 is identified to be expressed in HCC, gastric cancer and colon cancer. (iii) *Tumor-specific antigen*. This antigen is only expressed in tumor cells, not in any normal tissues. The TSA-9 antigen is expressed in lung cancer and colon cancer, whereas the TLH 6 antigen expressed in HCC only. (iv) *Cell differentiation antigen*. HCA519 is overexpressed in the vast majority of HCC specimens. CT11 cDNA is uniquely expressed in normal liver cells and HCC, its expression level is conversely correlated to the differentiation status of the HCC. In an attempt to elute the naturally processed and presented tumor antigenic epitopes from HCC by proteomics, we have identified a MAGE-A3 p271-279 peptide from the MAGE-3 mRNA+HLA-A\*0206+HCC cell line HLE. From the eluate of a resected HCC specimen, we have also identified the MAGE-A3 p271-279, NY-ESO-1 p157-165 and a novel MAGE-A1 p289-298, which is predicted to be presented by HLA-B\*0702.

The prerequisite condition to apply a tumor antigen vaccine is that patients have a positive immune response to tumor antigens. Application of the methods of peptide-MHC tetramer, IFN-gamma release ELISPOT/Cytospot, and granzyme B release ELISPOT assays, we have identified that the CD8 T-cell response specific to NY-ESO-1 p157-165, MAGE-A3 p271-279 and

HCA587 p317-325 are 34.8% (8/23), 42% (8/19) and 17% (2/12) positive, respectively, in HCC patients bearing the respective CT antigen encoding gene mRNA tumors and expressing HLA-A2 functional supertype. The functional supertype of HLA-A2 allelic molecules for the presentation of NY-ESO-1b peptide includes HLA-A\*0201, -A\*0203, -A\*0206, and -A\*0207. These HLA-A2 subtypes constitute nearly 100% of HLA-A2 alleles in the Chinese Han ethnic population. The HCA587 p317-325 is a novel peptide we have identified which is presented by HLA-A\*0201 molecules. The CT antigen HCA661 can also elicit specific CTL response in healthy donors.

The application of tumor antigens is not constrained to the preparation of tumor antigen vaccine. One of the applications is to determine the recurrence of the cancer and the prognosis of the patients. By nested RT-PCR, we have detected the MAGE-A1 and -A3 mRNA in the PBMCs of HCC patients prior to and after tumor resection. The survival time is short if the tumor antigen is persistently detectable in PBMCs during the follow-up after resection. In contrast, the survival time is much longer if the tumor antigen is undetectable in PBMCs during the follow-up after resection. We have also detected TSA-9 mRNA positive in 56% of the PBMCs of lung cancer patients with TSA-9 mRNA+ tumors. The FATE/BJ-HCC-2 mRNA was detected positive in 50% of the PBMCs of HCC patients.

Among the 200 tumor antigen encoding genes identified, several antigenic molecules may have a pivotal role in tumor biology. The novel CT antigen HCA661 is also identified as a new member of TFDP transcription factor family. HCA661 inhibits E2F 1-5 transcriptional activity and suppresses the expression of p53 through which to promote Lo cell growth, a liver cell line transfected with HCA 661 cDNA. The HCA520 we identified is expressed in a limited set of normal tissues and highly expressed in HCC but not in normal liver tissues. HCA520 is a novel calcineurin homologous protein (CHP2), which shares 54% homology at protein level with calcineurin subunit B. The HCA520 promoted the proliferation of 293 cells transfected with that gene's cDNA. The signaling pathway for the CHP2 promotion of cell proliferation is primarily characterized as: CHP2 → calcineurin phosphatase activity → nuclear translocation a special isoform of NFAT → activate endothelin 1 and EGFR, both are growth factors for 293 cells.

Collectively, we have cloned and identified several types of novel tumor-associated genes from HCC specimens; we have demonstrated that HCC patients have a cellular immune response to CT antigens. All these findings have the potential

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in vaccine design for immunotherapy, tumor prognosis, and understanding tumorigenesis through which to provide hints for drug design strategy.

We will further search for potent tumor antigens by proteomics and bioinformatics; continuation of the preclinical trial assays of the CT and CP antigens we have identified and the efforts to put them into clinical trials for immunotherapy in cancer patients; analysis of the characteristics of tumor microenvironment and the types of immune response of tumor patients to the tumor antigens for understanding protective immunity and tolerance; we will initiate to explore the novel immune response assay with high sensitivity and specificity in the attempt to reduce blood volume drawing from patients and make the assay to be feasibly performed in China.