HCA520, A NOVEL TUMOR ASSOCIATED ANTIGEN, INVOLVED IN CELL PROLIFERATION AND APOPTOSIS

YANG Mei-xiang 杨美香 [1], QU Xun 曲迅 [1], LIU Fu-li 刘福利 [2], ZHENG Guang-juan 郑广娟 [3]

[1] *Basic Research Institute of Clinic Medicine, Qilu Hospital, Shandong University, Jinan 250012;*
[2] *Shandong Provincial Hospital, Jinan 250000;*
[3] *Shandong University of Traditional Chinese Medicine, Jinan 250013, Jinan 250014*

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ABSTRACT

Objective: Tumor associated antigen encoding gene HCA520 (AF146019) was identified by screening a human hepatocellular carcinoma expressing cDNA library using SEREX technique. In this experiment we studied the effect of HCA520 on cell proliferation and apoptosis. Methods: Gene HCA520 was gained by PCR and transfected into 293 cells. The stable expression cells were obtained by G418 selection. The cell proliferation was measured by [^{3}H]-TdR uptake and apoptosis assay was measured by FACS. Results: Eukaryotic expression plasmid pcDNA3-HCA520 was constructed and its stable transfectants were obtained. Overexpression of HCA520 inhibited the cell proliferation and enhanced cell apoptosis after serum deprivation. Conclusion: HCA520 is a novel tumor associated antigen that can affect cell proliferation and apoptosis.

Key words: Tumor associated antigen; HCA520; Sable transfected

The identification, isolation and biochemical characterization of tumor associated antigen (TAA) has become, and continue to be the most extensively explored field. Tumor associated antigen encoding gene HCA520 (AF146019) was identified by screening a human hepatocellular carcinoma expressing cDNA library using SEREX (Serological analysis of Recombinant Expression cDNA Libraries) technique[1]. The full length of the HCA520 is 2396bp, which is composed of seven extrons and has an open reading frame of 591bp, encoding 196aa with two distinct EF-hand motif. The expected molecular weight is 23 kD. Similarity research has shown that HCA520 protein shares highest homology with calcineurin B homologous protein (CHP, 76% similarity and 61% identities). CHP is a regular protein that participates in regulating the Na+/H+ exchanger function as previous reported[2,3]. Therefore the HCA520 protein may associate with the Na+/H+ exchanger. Na+/H+ exchanger is involved in the tumor generation, development and metastasis[4, 5]. The activation of Na+/H+ exchanger is regulated by several factors. However there have been fear reports on direct regulator of the Na+/H+ exchanger. In this study, we mainly focused on the effects of overexpression of HCA520 on the cell bioactivity, including cell proliferation and apoptosis.

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[8]**Author** to whom correspondence should be addressed.
Phone: (0086-531)-6586998;
E-mail: qiluxqu@163.com
Biography: Yang Mei-xiang(1976-), female, master of medicine, The Institute of Basic Medicine, Shandong University Qilu Hospital, majors in tumor immunology.

MATERIALS AND METHODS

Bacterial, Plasmid and Cell Line

The JM109 was kept by our laboratory. Eukaryotic expression vector pcDNA3 was ordered from Promega. The 293 cell line was kept by our laboratory.

Restricted Enzymes and Other Reagents

The restricted enzymes, XhoI and T4 DNA ligase were ordered from Promega.

The Advantage enzyme was ordered from Clontech. The plasmid extraction and purification kit, PCR product purification kit and the lipsome transfection kit TransFast™ were ordered from Promega.

Construction of HCA520 Eukaryotic Expression Vector

The encoding region of HCA520 was amplified by polymerase chain reaction. The primers were designed using the Generunner3.04 software: forward, CTC CTC GAG ATG GGG TCG CGC AGC CAC (including XhoI site); reverse: TCT TCT AGA TCA CTT ATC GTC GTC ATC CTT GTA ATC CTT CAG GAT CCG GAT GCT C (include XbaI site and a Flag tag). The reaction was carried out with the template pGEM-T-HCA520 that included the open reading frame of HCA520 according to the following conditions: 94℃, 5min, followed by 30 cycles of 94℃, 20sec, 65℃20sec, 72℃, 1min, with a final extension of 10min at 72℃. The PCR product was identified by electrophoresis in 1% agarose gel, purified by Wizard PCR preDNA purification system according to the manufacturer's instruction and cloned into pcDNA3 vector at XboI and XhoI sites. The resulted clone was confirmed by sequencing.

Selection and Identification of the Stable Transfected Cell Line

293 cell line was transfected with HCA520 plasmid by liposome according to the manufacturer's construction. Cells stably expressing HCA520 were first selected by G418 and then single clones were selected by RT-PCR and Immunoblot. Immunoblot staining for HCA520-Flag in transfected 293 cells using the mouse anti-flag antisera and Horseradish peroxidase-conjugated anti-mouse IgG was performed essentially as described[6].

Proliferation Assay of the Stable Transfected Cells

The cell proliferation was measured by [^{3}H]-TdR uptake. The cells in 96-well plates were incubated in DMEM containing 10% NBC for 48h, and pulsed with 0.5uCi (18.5kBq)/well [^{3}H] TdR for 14-16h. Then the cells were harvested by trypsinization and collected onto 0.45mm membrane by Harvester (Harvester 96MachII). The radioactivity of intracellular [^{3}H]- TdR incorporation was counted by liquid-scintillator (Beckman Wallac).

Apoptosis Assay of the Stable Transfected Cells

The apoptosis was induced by culturing the cells in serum deprived media for 72h. Then cells were typsinized and resuspended in PBS containing 5μg/ml propidium iodide and Annexin-V at 10^5/ml on an Epics XL/MCL Coulter analyzer. The radioactivity was assessed at 488nm on 10000 cells by flow cytometric analysis.

Statistics

The statistics software of SPSS 10.0 was used for statistical studies, comparison of the means was done by One-Sample T test.

RESULTS

Construction of HCA520 Eukaryotic Expression Vector

We used the specific primers to amplify the encoding region of HCA520 by PCR technique and obtained an expected band in the agarose gel (Figure 1). The target gene was cloned into the linearized eukaryotic expression vector pcDNA3. The recombinant was identified with restricted enzyme digestion that produced a 600bp band (Figure 2). The confirmed clone was sequenced and compared with the HCA520 sequence in Genebank. No mutation was found.

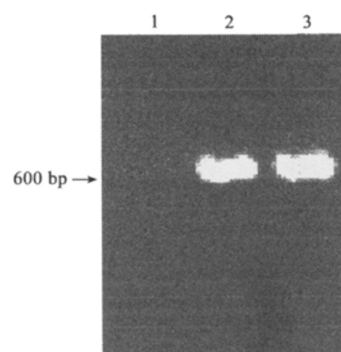

Fig. 1. PCR products of HCA520
1. Marker 2,3. PCR product

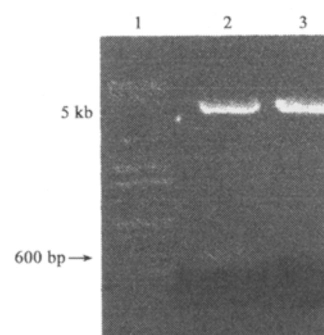

Fig. 2. Identification of PCDNA3. HCA520 by restriction enzyme digestion.
1. Marker 2. pcDNA$_3$-HCA520 3. pcDNA3

Transfection and Identification of 293 Cell Line

The transfected cells were selected by G418 and identified by RT-PCR and immunoblot. The results of RT-PCR were shown in Figure 3 there were two cell clones that expressed HCA520. The Western blot result was shown in Figure 4. There was an expected band for the cell lysate from pcDNA3-HCA520 transfected cells,