Bio-compatibility and cytotoxicity studies of water-soluble $CuInS_2$-ZnS-AFP fluorescence probe in liver cancer cells

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BACKGROUND: The oncogenesis of hepatocellular carcinoma (HCC) is not clear. The current methods of the pertinent studies are not precise and sensitive. The present study was to use liver cancer cell line to explore the bio-compatibility and cytotoxicity of ternary quantum dots (QDs) probe and to evaluate the possible application of QDs in HCC.

METHODS: $CuInS_2$-ZnS-AFP fluorescence probe was designed and synthesized to label the liver cancer cell HepG2. The cytotoxicity of $CuInS_2$-ZnS-AFP probe was evaluated by MTT experiments and flow cytometry.

RESULTS: The labeling experiments indicated that $CuInS_2$-ZnS QDs conjugated with AFP antibody could enter HepG2 cells effectively and emit intensive yellow fluorescence by ultraviolet excitation without changing cellular morphology. Toxicity tests suggested that the cytotoxicity of $CuInS_2$-ZnS-AFP probe was significantly lower than that of CdTe-ZnS-AFP probe (*t* test, F=0.8, T=-69.326, P<0.001). For $CuInS_2$-ZnS-AFP probe, time-effect relationship was presented in intermediate concentration (>20%) groups (P<0.05) and dose-effect relationship was presented in almost all of the groups (P<0.05).

CONCLUSION: $CuInS_2$-ZnS-AFP QDs probe had better bio-compatibility and lower cytotoxicity compared with CdTe-ZnS-AFP probe, and could be used for imaging the living cells *in vitro*.

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KEY WORDS: $CuInS_2$-ZnS quantum dot; HepG2 cells; bio-compatibility; cytotoxicity

Introduction

Hepatocellular carcinoma (HCC) is universally a common malignancy and the prognosis is poor.[1, 2] In 2010, the new HCC cases and death in China were 358 840 and 312 432, respectively.[3] Studies on HCC oncogenesis and progression are important to improve the outcomes of this population.

Quantum dot (QD) is an artificial nanoparticle (2-10 nm).[4] The excellent optical properties of QDs attract a lot of attentions from applications of medicine to bio-imaging field.[5] Fluorescence probe QDs have already been used in biological imaging *in vivo* and *in vitro*.[6-8] The surface of QDs can be functionalized by conjugating with polypeptide, protein and antibody and therefore, extraordinary combining capacity of QDs was observed in tumor targeting and imaging *in vivo*.[9-11] Originally the cadmium-series QDs were the most attractive in the cell fluorescent labeling due to their easily growth craft, but high cytotoxicity restricted its further application.[12] On the other hand, in order to seek a better QDs with higher optical stability, $CuInS_2$ and $AgInS_2$ QDs are fabricated and become the commonest free cadmium QDs with good bio-imaging capacity.[13] Although $CuInS_2$ and $AgInS_2$ are cadmium free QDs with a potential in biomedical applications, the literature on the synthesis, imaging of the cells and cytotoxicity in living cells are paucity. The aim of the present study was to expand the bio-function of the ternary QDs, via testing the cytotoxicity and bio-compatibility of the QDs probes labeling in the HepG2 cells.

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Alpha-fetoprotein (AFP) is considered a HCC marker.[14] In this study, AFP antibody was selected to conjugate the QDs $CuInS_2$-ZnS to form a new fluorescence probe $CuInS_2$-ZnS-AFP. So the probe could be used to combine and target the HepG2 cells, an HCC cell line that can positively express the AFP. The purpose of the study was to synthesize the probe, to detect the cytotoxicity and to investigate the application of the probe in liver cancer.

Methods

Materials

HepG2 cell line (from cell bank of Chinese Academy of Science), serum-free high glucose DMEM medium, fetal calf serum, PBS solution, penicillin-streptomycin, trypsin, dimethyl sulfoxide (DMSO), 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT), 4', 6-diamidino-2-phenylindole (DAPI), rabbit anti-AFP antibody, 3, 3'-diaminobenzidine (DAB) and secondary antibody were all purchased from Beijing Zhongshan Biotechnology Co., Ltd. Annexin V-FITC and leucocytic permeabilization reagent were purchased from American Beckman Coulter commercial enterprise Co., Ltd. $CuInS_2$-ZnS QDs synthesis related reagents were provided by University of Science and Technology of China.

Synthesis of $CuInS_2$-ZnS and CdTe-ZnS core-shell QDs

Hydrothermal synthesis method was used to synthesize $CuInS_2$ QDs. At room temperature, 0.15 mmol $CuCl_2$ and $InCl_3$ were added into 10.5 mL deionized water, then 1.8 mmol GSH was added into the solution. Solution PH was adjusted to 11.3 with 2 mol/L NaOH solution, 0.3 mmol thiourea was added and dissolved thoroughly by stirring the solution for 10 minutes. The $CuInS_2$-ZnS core-shell QDs were prepared by adding 0.8 mmol $Zn(OAc)_2 \cdot 2H_2O$, 1 mmol sodium citrate and 1.2 mmol GSH into the CZIS QD solution. Then 0.8 mmol Na_2S solution was slowly added. After stirring for 10 minutes, the reaction solution was heated for 2 hours at 100 ℃. The mixed liquor was transferred into a 15 mL autoclave and heated at 150 ℃ for 21 hours. After the solution was cooled to room temperature in cold water, ethanol was added, the precipitate was centrifuged with high-speed centrifuge and the pellet was dissolved in the deionized water. CdTe-ZnS core-shell QDs were provided by University of Science and Technology of China and the preparation was according to the instructions.[15]

Preparation of $CuInS_2$-ZnS-AFP and CdTe-ZnS-AFP QDs probe

100 μL of these two QDs solution (12.8 mmol/L) was added to the mixture solution which contained 10 μL 200 mg/mL EDC, 10 μL 20 mg/mL sulfo-NHS and 190 μL PBS. After 1 hour incubation at 37 ℃, PBS was added and the solution was centrifuged for 3 times, the supernatant was discarded and precipitate was resuspended by 600 μL PBS. 100 μL of 0.1 g/mL rabbit anti-AFP antibody was added and the reaction solution was shaking for 1 hour at room temperature, and after 3 times centrifuging QDs probe precipitate was resuspended in PBS. Both of the densities of these two QDs probes were 1.2 mg/mL.

Preparation of cell-coverslips

The exponential phase HepG2 cells were trypsinized and harvested. Sterile coverslips were placed in the six-well plate; cell suspension was dropped into each well with the density of $50\text{-}100\times10^3$/well and cultured in an incubator with 5% CO_2 at 37 ℃ for 24 hours.

Cell labeling and imaging

At room temperature, cell coverslips were washed for 3 times with PBS and 50 μL fixation agent was dropped and maintained on the glasses for 10 minutes. The cell glasses were washed as above and 50 μL permeability agent was added and washed after 5 minutes.

50 μL 0.24 mg/mL either $CuInS_2$-ZnS-AFP probe solution (experimental group) or $CuInS_2$-ZnS QDs solution (control group) was dropped onto the fixed cell glasses, respectively, and these glasses were placed in a humidified box and incubated at 37 ℃ for 40 minutes. These coverslips were observed under a fluorescence microscope (Olympus IX73) and a confocal laser scanning microscope (Leica SP5).

MTT assay

The suspension of exponential phase cells were harvested at the density of $5\text{-}10\times10^3$/100 μL, 100 μL cell suspension was planted in 96-well plates, the fringe wells were filled with 100 μL PBS. After 24 hours culture in incubator, all solution in these 96-well plates was discarded excepting for the fringe wells and two types of QDs probe solution with a range of concentrations (2.5%, 5%, 10%, 15%, 20%, 30%, 40% and 50%) was added, each concentration had five accessory wells, that were experimental groups. Control groups and zero setting groups were HepG2 cells+diluent and pure diluent, respectively. The diluent was high glucose DMEM medium with 10% fetal calf serum. After incubating with 5% CO_2 at 37 ℃ for 2 hours, 6 hours, 24 hours, 48 hours and 72 hours, cellular morphology was observed under microscope, then 0.5% MTT solution and DMSO were added. After that all the plates were shaken immediately and simulta-