ABSTRACT

Monoclonal antibody therapy for human epidermal growth factor receptor-2 (HER-2) is most effective in HER-2 positive breast cancer patient and is now widely used for treatment. Therefore, the early detection of HER-2 status in breast cancer patient is very important for the effective implementation of monoclonal antibody therapy. Recently, HER2 detections using antibody conjugated quantum dots (QDs) have attracted much attention. QDs are a new class of fluorescent materials that have superior properties such as high brightness, high resistance to photo-bleaching, and multi-colored emission by a single-light source excitation. QDs toxicity is a serious problem because it contains Cd$^{2+}$ ions. This study is divided into two part, 1) To assess in-vivo anti-HER2-CdSe QDs toxicity and 2) Development and in-vitro Bioimaging study using anti-HER2-CdSe-QDs. Apart from that, I have also studied in-vivo toxicity of silver nanoparticle (chapter-III). Silver nanoparticle is widely popular for medicinal use and also newly emerging non-toxic bioimaging probe.

In-vivo toxicity assessment is very important for QDs developed for bioimaging. Quantum dot conjugated with herceptin or anti-HER2 antibody is very recent fluorescent nanoprobe for HER2+ve breast cancer detection. In this study we have investigates in-vivo toxicity of CdSe/Cd(ZnS) quantum dot conjugated with anti-HER2 antibody and non-conjugated QDs. QDs toxicity assessment was carried out and explained in chapter-IIA. Wistar rat was selected as an animal model for this study and both male and female animal were used. Animals were randomly divided into control (n=6), anti-HER2ab-QDs (n=6), QDs (n=6) and reserve group (n=6). CdSe QDs were conjugated with anti-HER2 antibody using QDs antibody conjugation kit. QDs, anti-HER2ab-QDs and PBS were intravenously injected in animal and the parameter e.g., body weight, hematological, liver function enzyme assay, comet assay, ROS, TEM of liver and kidney, histology of organ tissue, apoptotic assay for liver and kidney cell and MTT assay were adopted for toxicity assessment. Genotoxicity was carried out by using comet assay from blood sample. Result showed that the complete blood count and biochemistry panel assay for liver function enzyme changed non-significantly in anti-HER2ab-QD treated group. EDXRF results were showed Cd deposition in non-coated QDs group but no such deposition was found in anti-
HER2ab-QDs treated group. There were no morphological changes found in organ histology and TEM imaging. Apoptosis was not detected in anti-HER2ab-QDs group compare to control. Animal treated with non-conjugated QDs show comet formation and apoptosis compare to the anti-HER2-QDs treated group. ROS and MTT assay for HEK293 cell treated with anti-HER2-QDs showed no significant different but significant changes was found in animal treated with QDs. To combine all findings, it is concluded that the anti-HER2ab-QDs in used concentration was non-toxic and no severe toxicity symptom was appeared. This study supports the biocompatibility of anti-HER-2-QDs for breast cancer imaging.

Chapter-IIB describes in-vivo bioimaging study of anti-HER2ab-QDs using HER2 over-expressed KPL-4 human breast cancer cell line. In this study, three types of anti-HER2-ab conjugated QDs (anti-HER2ab-QDs) was developed using various coupling agents (EDC/sulfo-NHS, iminothiolane/sulfo-SMCC, and sulfo-SMCC). At first, GSH-QDs were chemically synthesized and then conjugated with anti-HER2-ab and anti-CXCR4 ligand with different coupling method. GSH-QDs was highly emmissible and showed 0.23–0.39 quantum yield (QY) in aqueous solution. Dispersibility, hydrodynamic size, and apparent molecular weights of the GSHQDs and anti-HER2ab-QDs were characterized by using DLS, FCS, AFM and size-exclusion HPLC. Fluorescence imaging analysis of developed QDs for HER2 over expression was performed by using anti-HER2ab-QDs as fluorescent probes in KPL-4 cell line. HeLa cells were selected for control because these express normal level of HER2 receptor. We found that the anti-HER2ab-QDs prepared by using SMCC coupling with partially reduced antibody was most effective probe for the detection of HER2 expression in KPL-4 cells. These QDs also showed long time stability in liquid medium. Size dependent internalization of anti-HER2ab-QDs (540nm) was also observed in confocal fluorescence image of KPL-4 cells. In dual imaging analysis, QDs were conjugated with anti-HERab and anti-CXCR4 ligand through protein-A molecule. KPL4 cell treated with 5nM anti-HER2ab-Pro-A-QDs (650nm) and CXCR4-Pro-A-QDs (585nm). Imaging analysis was done by confocal fluorescence microscopy. By using this strategy, it was possible to target two different receptors at the same time for bioimaging analysis. These QDs can easily traced receptor on surface and other protein molecule inside the cells.
Apart from the QDs research, chapter-III, explored silver nanoparticle toxicity because silver is also among the widely used nanoparticle for medicinal purpose and newly emerging bioimaging probe. Study was performed to explore the effect of various doses of silver nanoparticle (AgNP) in rat. Four different doses of AgNP (4mg/kg, 10mg/kg, 20mg/kg and 40mg/kg) were injected intravenously for one month. For safety evaluation of injected AgNP, Body weight, organ coefficient, whole blood count and biochemistry panel assay for liver function enzyme (AST, ALT, ALP and GGTP), comet assay, ROS and histological parameter were adopted. 10-12 week old animal were randomly divided into control (n=6), 40mg/kg AgNP (n=6), 20mg/kg AgNP (n=6), 10mg/kg AgNP (n=6), and 4mg/kg AgNP(n=6). We observed significant changes (p<0.01) in hematogy parameter (WBC count, platelets counts, hemoglobin and RBC count) in high dose 40 mg/kg, 20mg/kg group but the changes was non-significant in low dose (4mg/kg and 10 mg/kg group). In 40mg/kg group, significant increase was also found in liver function enzyme like ALT & AST (p<0.01), ALP (p<0.01), GGTP (p<0.01), bilirubin (p<0.01). ROS in blood serum increased in high dose group. Tail migration in single cell gel electrophoresis in 40mg/kg 20mg/kg, 10mg/kg, 4mg/kg and control was 34.9μm, 29.5μm, 17.8μm, 5.8μm and 0.0μm respectively, which indicated damage in DNA strand in high dose group. Energy dispersive X-ray fluorescence spectroscopy (EDXRF) showed ~10 time increase in silver concentration in 40mg/kg group with respect to control. TEM image also showed particle deposition in 40mg/kg group. This study indicates that the AgNP in low doses (<4mg/kg) is safe for biomedical application and has very less side effects but the high dose (40mg/kg) cause some toxicity in animal body.