Targeting of Membrane Type1-Matrix Metalloproteinase (MT1-MMP) by using ultrasmall superparamagnetic iron oxide (USPIO) particles to hepatocellular carcinoma cells in vivo

Y. Chang[1,2], B-H. Park[2], J-C. Jung[3], Y-M. Lee[3], J-A. Park[2], H-J. Song[2], S-T. Woo[2], J-H. Kim[2]

[1]Diagnostic Radiology, Kyungpook National University Hospital, Daegu, Jung-gu, Korea, Republic of, [2]Biomedical Engineering, Kyungpook National University, Daegu, Jung-gu, Korea, Republic of, [3]Biology, Kyungpook National University, Daegu, Buk-gu, Korea, Republic of

Introduction

Membrane-type 1 matrix metalloproteinase (MT1-MMP) has been implicated in various physiological and pathological processes such as wound healing, bone development, angiogenesis, inflammation, rheumatoid arthritis, atherosclerosis, and cancer invasion and metastasis [1]. MT1-MMP is highly expressed in different cancers, and overexpression promotes migration, invasion and metastasis of cancer cells in vitro as well as in vivo. Therefore, the aims of this study is to verify tumor specificity of MT1-MMP at molecular biology level and to examine the possibility of MT1-MMP as a tumor marker using conjugation of streptavidinylated USPIO and biotinylated monoclonal antibody (mAb) MT1-MMP in MRI.

Material and Methods

Cell culture : The human colon cancer cell line HT-29 cells were cultured RPMI Medium 1640, supplemented with 10% heat inactivated fetal bovine serum(FBS) and antibiotics GIBCO^{TM}, Grand Island, U.S.A.). The human hepatoma cell line SK-HEP-1's were cultured Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10 % heat inactivated FBS and antibiotics. Each cells were cultured at 37℃, 5 % CO_2 incubator.

Western blotting and Immunofluorescence : Western blot analysis is undertaken using standard techniques. Specific antibody binding is visualized by enhanced chemiluminescence detection kit (Amersham, Biosciences) with X-ray film exposure. For intracellular staining the cells, the cells were permeabilized in 0.1 % Triton X-100 for 5 min. The specific MT1-MMP antibody was added to the wells and incubated for 1hr, followed by incubation with an Alexa 488-conjugated anti-mouse IgG for 30min. Images were viewed and photographed with a fluorescence microscope.

Tumor Model : Xenotransplanted nude mice of the BALB/c Slc-*nu* strain (Japan SLC., Inc.) were used as tumor model (male, 20-25 g body weight). SK-HEP-1 cells and HT-29 cells were transplanted in the lower flanks of the left and the right, respectively (3×10^6 cells, s.c.). All animal protocols approved by the institutional animal review board.

Conjugation of USPIO and mAb MT1-MMP (USPIO-MT1-MMP) : Monoclonal anti-human MT1-MMP ectodomain antibody(R&D Systems, Inc., Minneapolis) was dialyzed using biotin labeling buffer. N-hydroxysuccinimide ester biotin(10 ㎕ of 10 ㎎/㎖ DMSO) was added to antibody, and then incubated 1hr 30min at room temperature. After removing unbound biotin by dialysis, biotinylated mAB MT1-MMP was conjugated with lyophilized USPIO (1 ㎖ of lyophilized USPIO agent powder/30 ㎕ of Phosphate Buffered Saline). The USPIO's were obtained from commercial sources (Miltenyi Biotec, Auburn, CA). These agents consist of an iron crystal (~10 ㎚ diameter) coated with polysaccharide resulting in a ~50 nm diameter particle.

MRI : After anesthesia, nude mice with 10 ㎜ tumor diameter were imaged at 1.5 Tesla MR unit with transmit-receive birdcage human extremity coil. MRI was performed using T2 fast spin echo (FSE) (TR = 2500 ms, TE = 105 ms, number of excitations [NEX] = 1, matrix = 147*256, FOV = 108 mm).

Histological analysis **:** After MRI, the part of the nude mouse tumor was subjected to histological evaluation for prussian blue staining to detect USPIO-MT1-MMP labeled cells.

Results

MT1-MMP was detected as pro form (60 kDa) and active form (50 kDa) in SK-HEP-1 cells by western blotting. The result of immunofluorescence shows that MT1-MMP was strongly stained in SK-HEP-1 cells. In case of HT-29 cells, MT1-MMP was weakly stained [Fig. 1]. After injection of USPIO-MT1-MMP, MR signal intensity was not changed significantly in HT-29 cells (before injection; 187.7±13.7, after injection; 197.4±16.3). However the signal reduced from 189.3±46.3 to 78.6±6.8 in SK-HEP-1 cells compared with the image before injection [Fig. 2]. Histological analysis shows that SK-HEP-1 cells related nude mouse tumor stained with prussian blue [Fig. 3]. However, no USPIO particles were observed in HT-29 cells.

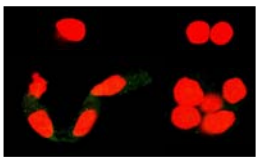

Fig. 1. Localization of MT1-MMP in HT-29 and SK-HEP-1 cells. Double-staining for MT1-MMP and PI (nuclear staining) staining

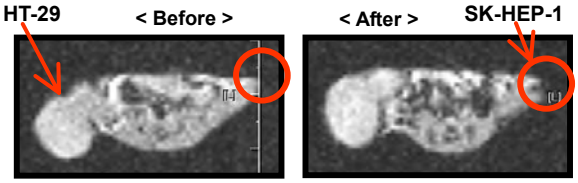

Fig. 2. MRI experiment of a nude mouse with subcutaneous HT-29 (left) and SK-HEP-1 tumor (right) before and after administration of USPIO-MT1-MMP. Left; before administration. Right; 30 min post injection

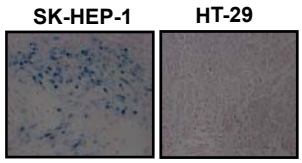

Fig. 3. Photomicrographs of prussian blue staining of HT-29 (right) and SK-HEP-1 (left) cells related tumors.

Discussion

Among the various tumor-associated antigens, membrane-type 1 matrix metalloproteinase (MT1-MMP) acts on the cell surface. MT1-MMP has been detected in tumor cells and adjacent stromal cells in variety of human tumors, including breast, cervical, colon, bladder, gastric, pancreatic, ovarian, prostate and thyroid cancer. MT1-MMP has important roles not only in cancer invasion but in tumor progression overall and acts on the cell surface. Up to our knowledge, it is a new trial that MT1-MMP was used as *in vivo* MR imaging target. Our data shows that MT1-MMP was detected in SK-HEP-1 (human hepatoma cell line) cells but there is only weak pro form band in HT-29 (human colon cancer cell line) cells. Along with these data, the result of immunofluorescence implies that MT1-MMP was strongly stained in SK-HEP-1 cells. However, MT1-MMP was weakly stained in HT-29 cells. From these in vitro assays, it was confirmed that MT1-MMP shows molecular specificity to human hepatocellular carcinoma cells. Finally, our *in vivo* MR experiment suggests the possibility of MT1-MMP as a tumor marker using conjugation of streptavidinylated USPIO and biotinylated mAb MT1-MMP (USPIO-MT1-MMP) in MRI. That is, it was successfully demonstrated that MRI can monitor grafted hepatocellular carcinoma (SK-HEP-1) by using MT1-MMP-USPIO system. Also, our results seem to suggest that the application of MMP to MR imaging target will contribute to the deeper understanding of molecular biology of MMP activity *in vivo*.

[1] Yoshifumi Itoh and Motoharu Seiki. TRENDS in Biochemical Sciences 29(6):285-289, 2004.