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Nanomedicine: Nanotechnology, Biology, and Medicine
xx (2015) xxx–xxx
nanomedicine
Nanotechnology, Biology, and Medicine
nanomedjournal.com

A multifunctional peptide for targeted imaging and chemotherapy for nasopharyngeal and breast cancers

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Received 24 June 2014; accepted 25 March 2015

Abstract

The L-peptide plays a role as a universal ligand binding specifically to nasopharyngeal carcinoma (NPC) and other cancers but not normal cells. It was used to link iron oxide nanoparticles, and injected intravenously to SCID mice bearing NPC and breast cancer xenografts for MR analysis, and showed significant change of MR signal intensity in the xenograft regions. Using this conjugate as a ligand to localize the L-peptide targeted protein in the cancer surgical specimens, a clear reaction product was identified in the tumor cells of both cancer types. If the L-peptide-linked-liposomal doxorubicin was used to treat the SCID mice bearing other NPC or breast cancer xenograft, a high efficacy of chemotherapy with minimal adverse effect was observed. In conclusion, the L-peptide has a considerable potential for clinical usage for targeted imaging, peptide histochemical localization of targeted protein, and targeted chemotherapy for different cancer types.

Key words: Nasopharyngeal carcinoma; Breast cancer; L-peptide; Peptide histochemistry; Peptide-targeted MRI and chemotherapy

In treating cancer with chemotherapy, the ultimate goal is to deliver sufficient amounts of the drug to tumor cells while minimizing the damage of normal tissue. Using a phage-displayed random peptide library to screen the nasopharyngeal carcinoma[1,2], we identified a specific peptide (L-peptide), which binds specifically to NPC tumor cells but not normal cells[3]. When this L-peptide (L-P) was linked to the pegylated liposomal doxorubicin (L-D), it shows a high efficacy in the treatment of NPC-TW01 xenografts in SCID mice with minimal adverse effects[3]. Using the similar strategy to search for other specific peptides for targeted chemotherapy in other cancers, such as lung cancer[4–6], hepatoma[7], glioblastoma[8,9], and bladder tumor[10], has

Abbreviations: NPC, nasopharyngeal carcinoma; NPC-TW01,06 and 07, nasopharyngeal carcinoma, Taiwan, cell line-01, 06 and 07; C-P, control-peptide; L-P, L-peptide; Dox, doxorubicin; L-D, liposomal doxorubicin; L-P-L-D, L-peptide-linked liposomal doxorubicin; C-P-L-P, control-peptide-linked liposomal doxorubicin; Fe_3O_4, iron oxide; Dex-Fe_3O_4, dextran coated iron oxide; L-P-Dex-Fe3O4, L-peptide-linked dextran coated iron oxide; C-P-Dex-Fe3O4, control-peptide-linked dextran coated iron oxide; FITC-C-P, fluorescein isothiocyanate-labeled control-peptide; FITC-L-P, fluorescein isothiocyanate-labeled L-peptide; FITC-L-P-Dex-Fe3O4, fluorescein isothiocyanate-labeled L-peptide-linked dextran coated iron oxide; B-C-P, biotin-control-peptide; B-mL-P, biotin-modified L-peptide; mL-peptide, modified L-peptide; BCL-2, B cell lymphoma-2; RGD peptide, arginylglycylaspartic acid peptide; MRI, magnetic resonance imaging; SCID mice, severe combined immunodeficiency mice; X-protein, unidentified protein; PBS, phosphate-buffered saline; NSCLC, non-small-cell lung carcinoma; emu, electromagnetic unit

Role of funding source: This study was supported in part by a research grant from National Taiwan University Hospital (NTUH 99-A102 to CTL and JKH), and by the grants from National Science Council (NSC97-2323-B-002-004, NSC100-2325-B-002-034, NSC101-2325-B-002-030 and NSC102-2325-B-002-029 to CTL; and NSC97-2314-B-303-018 MY 2 to JKH).

Competing interests: There are no competing interests to report.

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http://dx.doi.org/10.1016/j.nano.2015.03.011
1549-9634/

also been reported; similarly, peptides for targeted chemotherapy have also been developed from hormonal fragment to bind to their receptor expressed tumor cells[11]; using the analogs of peptide hormones for targeted receptors on tumors has also been described[12]; in addition, synthetic BCL-2 homology 3 domain peptide[13], apoptosis-targeted peptide[14] for targeted chemotherapy, and RGD or chlorotoxin peptide for tumor targeting ligands, have also been published[15].

The imaging of cancer cells is diagnostically and therapeutically important[15]. Molecular imaging could fulfill the needs of medical imaging[16,17]. Iron oxide (Fe3O4) nanoparticles are ideal for molecular imaging. The particles are composed of Fe3O4 core with a biodegradable coating, such as dextran or carboxydextran[18]. The iron core could be metabolized and re-synthesized as hemoglobin[19]. Furthermore, the Fe3O4 nanoparticles are very sensitive compared with traditional gadolinium-based magnetic resonance imaging (MRI) contrast medium[18]. Moreover, the nanoparticles could be modified at the surface to allow important biomedical molecules, such as peptides, to link to them and could be used as a sensitive probe for targeting important biomedical[15,17]. In the present experiment, we have linked the L-peptide to Fe3O4 nanoparticles as a targeted guide for molecular imaging analysis of NPC xenografts by MRI.

To verify whether the tumor cells in other undifferentiated NPC and other cancer cell types could also be bound by the L-peptide and whether the L-P-L-D-treated SCID mice bearing other undifferentiated NPC and breast cancer xenografts could also reveal marked tumor shrinkage while maintaining normal histological features of visceral organs, we planned to develop a new technology to localize the peptide targeted X-protein, since the regular biotin labeled peptide could poorly or not bind to the formalin-fixed paraffin-embedded surgical specimens.

Methods

Cell lines, biopsy and surgical specimens, animals, peptides

For details, please see the supplementary methods.

Flow cytometry analysis of L-peptide binding cells in NPC and other cancer cell lines by FITC-L-peptide

For details, please see the supplementary methods.

Localization of the L-peptide-targeted protein in NPC and breast cancer cell lines and their surgical specimens.

For details, please see the supplementary methods.

Localization of L-peptide targeted protein by L-phage

For details, please see the supplementary methods.

Synthesis and characterization of magnetic nanoparticles (The dextran coated-Fe3O4 was linked with L-peptide by the MagQu Company)

For details, please see the supplementary methods.

MRI analysis of iron oxide nanoparticles in the test tube and NPC cells

For details, please see the supplementary methods.

MR imaging analysis for NPC and breast cancer cells in vitro and in xenografts in animal experiment

For details, please see the supplementary methods.

Quantitative MRI for cancer xenograft size analysis and statistical analysis

For details, please see the supplementary methods.

Efficacy of L-peptide-linked liposomal doxorubicin (L-P-L-D) treatment for NPC and breast cancer xenografts in SCID mice

Previously, we used L-P-L-D to treat NPC-TW01 (a keratinizing squamous cell carcinoma) xenografts in SCID mice and obtained very promising results[3]. To ascertain whether L-P-L-D is also effective for other undifferentiated NPC cell line and breast cancer cell line, we used the procedure similar to the previous publication[3]. Briefly, the SCID mice were transplanted with 1×10^7 NPC-TW06 cells into the left flank or right thigh. After 14 days, solid xenografts measuring approximately 0.8 cm in diameter were found in each mouse. Then, they were divided into three groups (each group included 5 animals). One group was injected with PBS; the other two groups were injected either with C-P-L-P or L-P-L-D. Each mouse was injected through the tail vein with 1 mg of L-D per kg of mouse body weight, once per week, for a total of 5 injections, and all animals were sacrificed at day 49. The body weight and tumor size were measured weekly. All were examined by routine autopsy. The xenograft tumors and all visceral organs were examined by histopathological sections after staining by hematoxylin and eosin. The pegylated L-D was a gift from the TTY Biopharm Co. Ltd. in Taipei, Taiwan. The L-P and C-P after pegylation were linked to L-D in our laboratory according to our previously published method[3]. Similarly, we transplanted MDA-MB-231 cells into SCID mice at the left thigh. For comparison, the animals were divided into 4 groups. The first group was treated with PBS, and 2nd, 3rd and 4th groups were treated with free Dox (in powder form), C-P-L-D, or L-P-L-D, respectively. Each of 2nd to 4th groups was injected with 1.2 mg of Dox per kg of mouse body weight, once per week, for a total of 4 weeks. The animals were sacrificed one week after the last injection. The animal body weights, the xenograft tumor weights, and the visceral organs were examined as usual.

Results

Flow cytometry analysis of the L-peptide binding cells in different cancer cell lines

When the NPC-TW07 line was incubated with the FITC-L-P, a clear peak was found in the histogram (Figure 1, *A*). It is significantly different from NPC cells incubated with FITC-C-P. When other NPC lines including NPC-TW01 and other cancer lines including NSCLC (A549 and H1299 cell line), neuroblastoma (Be2C), and breast cancer (MDA-MB157) were incubated with the FITC-L-P, all showed a clear binding peak that was different from the immortalized renal cell line (293 T cell) (Figure 1, *B*). A histogram analysis from the flow cytometry analysis in Figure 1, *B* has been shown in Figure 1, *C*. The *P* value revealed a significant difference (* $P < 0.05$; ** $P < 0.01$).

The fold increase for the fluorescent intensity in cancer cell compared to 293 T line is shown in Figure 1, *C*. The fold increase is statistically significant. To be sure that the untransformed 293 T cell line cannot be bound by L-P, we have incubated L-P-Dex-Fe_3O_4 with 293 T culture cell and shown no binding of L-peptide on the 293 T cell surface (Figure 1, *D, b*); further, if the culture cells were