The Use of Ligand Conjugated Superparamagnetic Iron Oxide Nanoparticles (SPION) for Early Detection of Metastases

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BACKGROUND

Breast cancer is the most common cause of cancer death in women; more than 75 % of patients die from skeletal metastases [1]. The accurate diagnosis of metastatic disease is therefore crucial for treatment and survival. A number of breast cancer cells express receptors for luteinizing hormone releasing hormone (LHRH) and luteinizing hormone (LH) [2,3]. We previously demonstrated that human breast cancer tumors and their metastases can be targeted through their LHRH and LH receptors [3].

Sensitive, non-invasive methods for early detection of metastases need to be developed for accurate diagnosis to improve and monitor treatments to increase the survival of the patients. The sensitivity of magnetic resonance imaging (MRI) can be increased by use of superparamagnetic iron oxide nanoparticles (SPION) as a contrast agent. Sufficient intracellular accumulation of iron particles is required to achieve the maximum resolution in MR imaging.

Intravenously injected SPIONs accumulated mainly in the liver. Therefore MR imaging using SPION as contrast agent was applied for detecting liver metastases. An accurate estimate of liver metastases of greater than 1 cm in diameter was achieved [4]; however, detection of smaller lesions was unsatisfactory [5]. Detection of metastatic cells in the peripheral organs and bones remains difficult because of unsufficient accumulation of iron particles in the cancer cells.

We hypothesized that targeted delivery of SPIONs may specifically facilitate a concentration of particles at the tumor/metastatic cells, and an incorporation through receptor mediated endocytosis, resulting in increased accumulation of SPION in metastatic cancer cells of peripheral tissues, lymph nodes and bones. Sufficient SPION concentration in the cancer cells may increase the sensitivity of MR imaging. In this study we tested whether LHRH-SPION specifically target and accumulate in metastatic cells from breast cancer xenografts, and whether the resolution of MR images can be increased.

METHODS

SPIONs were fabricated as described in Kumar et al [6] using wet chemical methods and then conjugated to LHRH by carbodiimide reactions [6].

Female nude mice bearing human breast cancer xenografts (MDA-MB-435S.luc) were injected with LHRH-SPION or SPION into the lateral tail vein. Twenty hours after injection the mice were sacrificed and the organs and tumors were collected. Metastatic cells in peripheral organs and bones were determined by luciferase assays from homogenates. The iron contents were determined spectrophotometrically by Prussian blue reaction from organ homogenates and from paraffin embedded tissues. The following groups (8 mice each) were investigated: Tumor bearing mice with 1. saline injections, 2. with SPION injections, 3. with LHRH-SPION injections, and normal mice (no tumor) 4. with and 5. without SPION injection.

RESULTS

1. The LHRH-SPIONs were nearly monodisperse with a mean diameter of 10 nm. They did not agglomerate and were comparable to SPION alone.
2. Both SPION and LHRH-SPION remained super-paramagnetic before and after binding to LHRH
3. LHRH-SPIONs were directly incorporated within the cancer cells of the primary tumors and disseminated cells from peripheral tissue.
4. Unconjugated SPIONs accumulated largely in the liver, showed poor affinity to the tumor and were not detectable in metastatic lesions.
5. LHRH-SPION accumulated at higher concentration in the tumor cells than SPION alone and resulted in a 7.5 fold higher iron concentration in tumors and approximately 100 fold higher iron concentration in metastatic cells from lungs compared to free SPION.
6. The amount of LHRH-SPIONs accumulated in lungs was directly dependent on the number of metastatic cells in the same organ.

7. Transmission electron microscopy showed that LHRH-SPION accumulated in cytoplasm and the nuclei of the target cells.
8. LHRH-SPION built clusters of particles within the tumor cells
9. Only LHRH-SPION increased the contrast of the MR image

CONCLUSION

LHRH-SPION accumulated in cancer cells and their metastases directly, specifically and increased significantly the intracellular iron concentration. LHRH-SPION may serve as a contrast agent in MRI for early detection of metastases and disseminated cells and increase the sensitivity and image resolution.

Keywords: metastases, superparamagnetic iron oxide nanoparticles, luteinizing hormone releasing hormone receptors, magnetic resonance imaging

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