Magnetic iron microbeads coupled with HEA-125 monoclonal antibody against epithelial cell adhesion molecule

EpCAM microbeads

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Chemical name:	Magnetic iron microbeads coupled with HEA-125 monoclonal antibody against epithelial cell adhesion molecule	
Abbreviated name:	EpCAM microbeads	
Synonym:	CD326 microbeads	
Agent Category:	Nanoparticles	
Target:	Epithelial cell adhesion molecule (EpCAM)	
Target Category:	Adhesion molecules	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal / contrast:	Iron oxide	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available.

Background

[PubMed]

Magnetic iron microbeads coupled with HEA-125 monoclonal antibody against the epithelial cell adhesion molecule (EpCAM), abbreviated as EpCAM microbeads, have been developed primarily for the positive selection or depletion of EpCAM-positive cells (1, 2). McClelland et al. have demonstrated the feasibility of cell tracking with magnetic

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resonance imaging (MRI) after the hepatic progenitor cells are labeled with EpCAM microbeads (1).

Superparamagnetic iron oxide (SPIO)-based agents have been intensively tested for use in cell tracking with MRI in both preclinical and clinical situations (3, 4). SPIO particles provide a strong change in signal per unit of metal in T2-weighted images without a significant effect on labeled cells and host (3, 5). For efficient cell labeling, SPIO particles are generally coated with low-molecular-weight polymers or dendrimers leading to clusters of electron-dense crystal cores covered with the polymer or dendrimer. Surface coating increases the stability of SPIO particles and allows further chemical modification of the particles with targeting ligands. Cell tracking studies have shown that the migration and homing capabilities of SPIO-labeled cells can be monitored *in vivo* over days to months (3, 4, 6).

Nevertheless, there are still many challenges to overcome before MRI cell tracking can be considered a robust technique in preclinical settings or in clinical applications (3, 7). MRI detects the presence of SPIO contrast agents, regardless of whether SPIO particles remain in the relevant cells, are lost to the extracellular matrix, or are transferred to other cells. It is still not possible to use MRI to discriminate live cells from dead cells or relevant cells from phagocytes. Detection sensitivity also becomes an issue when cells actively divide and migrate, in which case the SPIO labels are quickly divided among daughter cells to levels that are undetectable with MRI. I*n vivo* quantification of the cell number is more challenging because of the contrast agent dilution during cell division, contrast agent transfer to others cells, other sources of iron in tissue, and technical limitations (4, 7).

McClelland et al. addressed the problem of contrast agent dilution in MRI cell tracking by using EpCAM microbeads as the label (1). The human EpCAM, also known as CD326 or epithelial-specific antigen, is a cell-surface antigen and is found on hepatic progenitor cells, including human hepatic stem cells (hHpSCs) and hepatoblasts (hHBs), on liver cancer stem cells, and on proliferating epithelial cells in other tissues (8-10). The human EpCAM is not expressed in animal cells. McClelland et al. studied the labeling of hHpSCs with EpCAM microbeads *in vitro*, *ex vivo*, and *in vivo* and imaged the labeled cells with MRI (1). The investigators demonstrated that the hHpSCs could be labeled with EpCAM microbeads before or after transplantation, and the transplanted hHpSCs could be monitored and counted repeatedly in the same host by injection of the label just prior to MRI (1).

Related Resource Links:

- Chapters on MRI cell tracking in MICAD
- The protein and nucleotide information of EpCAM
- Articles on EpCAM in Online Mendelian Inheritance in Man (OMIM)
- Datasheets of the CD326 MicroBeads

Synthesis

[PubMed]

EpCAM microbeads (brand name, CD326 MicroBeads) are commercially available and have an overall diameter of 50–100 nm (1). The magnetic iron microbeads were coupled with HEA-125 monoclonal antibodies against the human EpCAM. The amount of monoclonal antibodies per microbead was not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Cell labeling with EpCAM microbeads was analyzed after the hHpSCs, prepared from human fetal livers, were exposed to the beads at a concentration of 10 µl EpCAM microbeads per 10^7 cells in 100 µl buffer for 40 min at 4°C (1). After labeling, excess microbeads were washed, and the cells were cultured for 24 more hours. Transmission electron microscopy, scanning electron microscopy, and dispersive x-ray imaging confirmed the cell internalization and cell surface attachment of the EpCAM microbeads. The labeling efficiency. i.e., the amount of Fe or beads per cell, and the effect of labeling on cell function were not reported.

Detection feasibility with MRI was tested with labeled and unlabeled cell aggregates embedded in 1% agarose on 35-mm Petri dishes (1). In contrast to the controls without cells and with unlabeled cells, gel that contained labeled cells showed conspicuously large clusters of signal voids of varying sizes, which were attributed to the cell aggregates of varying sizes. Detection sensitivity was not reported.

McClelland et al. established an MRI method for both *in vitro* and *in vivo* quantification of the cell number (1). Three-dimensional aggregates of the hHpSCs were generated by placing cells in culture dishes. The radius, diameter, and area of the cell aggregates were measured under microscopy as well as with MRI, assuming that a single hHpSC has a radius of ~8 µm and that the descendents of hHpSCs (the hHBs) have a single-cell radius of ~11 µm. A strong correlation ($r^2 = 0.99$) was obtained between the cell aggregate radii obtained under microscopy and those measured with MRI. The cell aggregates appeared larger (64% larger in this case) in MRI than in actuality because the influence of the magnetic beads that give rise to the MRI contrast usually extends beyond their physical boundary. On the basis of the cell number and cell aggregate radius measured under microscopy, equations for the cell count (N) in terms of MRI-observed aggregate radius (r_{MRI}) were then established as $N \approx 104 + 4.2E - 4r^3_{MRI}$ for spherical aggregates and $N \approx 104 + 1.0E - 4V_{MRI}$ for non-spherical aggregates, where the E represents the power of 10 and the V_{MRI} is the volume of the MRI region of interest. Cell numbers could be computed directly from the *in vivo* MRI images with the use of these equations (1).

Animal Studies

Rodents

[PubMed]

McClelland et al. first tested the MRI detection and quantification of labeled cells *in situ* with a 7-T scanner (1). Livers from Sprague-Dawley rats (n = 3) were isolated and perfused with buffer. Labeled and unlabeled hHpSCs (~2×10^6) were transplanted directly into the livers through portal vein catheterization, and the organs were imaged immediately after transplantation. Intensity voids from the aggregates of labeled cells were observed throughout the liver. The labeled cell aggregates had an average radius of 133.4 μm, which corresponded to an average cell count of 1.1×10^2.

McClelland et al. then analyzed the post-transplantation cell labeling efficiency and quantified the cell number (1). Labeled and unlabeled hHpSCs (2×10^6) were injected into the liver of SCID/NOD mice (n = 16) through the splenic vasculature that is connected directly to the liver. In this way, the majority of the cells went directly to the liver. Eight hours later, the mice were euthanized, and the livers were excised, perfusion-fixed, and imaged. For the controls injected without labeled hHpSC cells, injected with microbeads alone, and injected with unlabeled hHpSCs (n = 4 mice/group), MRI images of the perfused liver appeared as a homogeneous background except for the hyperintense branching pathways near the liver center that correspond to the hepatic sinusoids. For the mice injected with unlabeled hHpSCs but subsequently given microbeads through the tail vein on days 6 and 18 after transplantation, a total of four and seven hHpSC aggregates were detected as hypointense foci in the MRI image slices from days 6 and 18, respectively. In the image slice from day 18, the cell aggregates were clearly located near the large sinusoidal pathways of the liver, which is consistent with the fact that cells transplanted into the liver *via* the portal vein migrate through sinusoidal pathways before integrating into the liver tissue. The number of cells in the aggregates was calculated with the equations described in *In Vitro* Studies. More aggregates and up to 12 times more cells were found on day 18 than on day 6, indicating post-transplantation cell proliferation.

In summary, McClelland et al. demonstrated a method for *in vivo* MRI cell tracking that enabled noninvasive monitoring of hHpSCs after transplantation (1). The cells could be labeled before or after transplantation with EpCAM microbeads that can distinguish transplanted cells from host cells. In this study, however, the background in MRI imaging was limited by using xenogeneic transplants of human hepatic progenitors into animals, by using EpCAM antigen expressed only on the human cells, and by perfusing liver tissues to remove blood before imaging. The accuracy of the cell number quantification from the *in vivo* images was not further validated (1).

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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