Video Article

In vitro Labeling of Human Embryonic Stem Cells for Magnetic Resonance Imaging

Mayumi Yamada[1], Phillip Yang[1]

[1]Division of Cardiovascular Medicine, Stanford University

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Abstract

Human embryonic stem cells (hESC) have demonstrated the ability to restore the injured myocardium. Magnetic resonance imaging (MRI) has emerged as one of the predominant imaging modalities to assess the restoration of the injured myocardium. Furthermore, ex-vivo labeling agents, such as iron-oxide nanoparticles, have been employed to track and localize the transplanted stem cells. However, this method does not monitor a fundamental cellular biology property regarding the viability of transplanted cells. It has been known that manganese chloride ($MnCl_2$) enters the cells via voltage-gated calcium (Ca^{2+}) channels when the cells are biologically active, and accumulates intracellularly to generate T^1 shortening effect. Therefore, we suggest that manganese-guided MRI can be useful to monitor cell viability after the transplantation of hESC into the myocardium.

In this video, we will show how to label hESC with $MnCl_2$ and how those cells can be clearly seen by using MRI in vitro. At the same time, biological activity of Ca^{2+}-channels will be modulated utilizing both Ca^{2+}-channel agonist and antagonist to evaluate concomitant signal changes.

Video Link

The video component of this article can be found at http://www.jove.com/video/827/

Protocol

Manganese Labeling of Human Embryonic Stem Cells

1. Trypsinize the human embryonic stems which have been cultured in feeder-free conditions for 5 minutes. Neutralize the trypsin by adding culture media.
2. Spin down the cells by centrifuging at 800 rpm for 5 minutes at 20° Celsius. After re-suspending the cell pellet, count the number of cells by trypan blue staining. Based on the cell count obtained, divide the cells into four aliquots of 3 million cells into conical tubes. Pellet the cells again by centrifugation.
3. Just before starting the manganese labeling, dissolve fresh manganese chloride with 0.9% sodium chloride solution to make a 0.1mM manganese chloride solution.
4. In order to observe calcium channel activity, four samples were prepared. The first sample is our control which contains cells incubated in 0.9% sodium chloride alone. The second sample contains cells incubated in 0.1 mM $MnCl_2$. Sample 3 and 4 contain cells incubated in 0.1 mM $MnCl_2$ with either 5 μM of the Ca^{2+}-channel agonist, (s)-(-)-Bay K8644, or 250 μM of the Ca^{2+}-channel antagonist, verapamil. Incubate the four samples at 37° Celsius with 5% CO_2 for 30 minutes.
5. After 30 minutes, spin down the cells at 800 rpm for 5 minutes at 20° Celsius. Then aspirate the supernatant and wash the labeled cells twice with PBS. After washing, re-suspend each pellet with 200 μL PBS and transfer into PCR tubes. These pellets are usually white.

Iron Labeling of Human Embryonic Stem Cells

1. Mix clinical-grade protamine sulfate with distilled water to get a 1 mg/mL stock solution.
2. In a tube containing human embryonic stem cell culture media, add ferumoxides at 100 μg/mL followed by addition of 12 μg/mL protamine sulfate. Mix the solution vigorously for five minutes.
3. Add mixed solution to the equal amount of the cell culture media to achieve a final concentration of 50 μg/mL of ferumoxides and 6 μg/mL of the protamine sulfate to label cells. Incubate cells in this solution for 12 hours.
4. Wash the cells twice with PBS, with the last wash containing 10U/ml heparin to dissolve the extracellular ferumoxides-protamine sulfate complex. After washing, trypsinize cells to obtain a single cell suspension.
5. Count the cells and aliquot into the required number of samples. After washing, re-suspend each pellet with 200 μL PBS and transfer into PCR tubes. These pellets should be dark orange brown in color.

Performing Cellular Magnetic Resonance Imaging

1. Make a phantom in order to stabilize the tubes containing the cell pellets and also to avoid artifacts from the surrounding air during the scanning. To do so, mix 0.8% of agar and 1% of copper sulfate in distilled water and bring it to a boil by microwaving for 5-7 minutes. Cool this mixture for at least 1 hour prior of scanning to attain a gel.
2. The tubes containing the labeled cell pellets are placed in the phantom for scanning. In vitro cellular MRI is performed at Signa HDx 3T MRI (GE Medical Systems) by using clinical knee coil.
3. Scan the manganese labeled cells for each of the three samples using a spin echo sequence: TR; Repetition time = 800 msec. TE; Echo time = minimum, FOV; field of view = 12 x 12 cm; matrix = 256x256, NEX 1.
4. Scan the iron labeled cells by using a gradient-echo sequence. We use the following parameters for this imaging: TR = 100 msec; TE = 10 msec, FA; flip angle = 30°; FOV = 12 x 12 cm; matrix = 256 x 256, NEX 1.

Analyzing the MRI Results

1. Analyze images of MRI scanning from 4 different samples of manganese-labeled cells:
   - Sample #1 is our control which contains cells incubated in 0.9% sodium chloride alone.
   - Sample #2 contains cells incubated in 0.1 mM $MnCl_2$.
   - Sample #3 contains cells incubated in 0.1 mM $MnCl_2$ with 5 µM of the Ca^{2+}-channel agonist, (s)-(-)-Bay K8644.
   - Sample #4 contains cells incubated in 0.1 mM $MnCl_2$ with 250 µM of the Ca^{2+}-channel antagonist, verapamil.

2. As predicted, differences in signal intensities from manganese labeled cells are visible. Compared to the cells labeled purely with manganese, the signal intensity clearly increases in the presence of the calcium channel agonist and decreases in the presence of calcium channel antagonist. The area where signal intensity has increased for manganese labeled cells are measured by using Image J (NIH, Bethesda, MD, USA). The same software is used for analyzing the area of dark off-resonance signal from iron labeled cells. The size of the dark signal from the iron-labeled cells depends on the number of cells or the number of iron particles contained by cells. In this case, one million cells shows a smaller area of dark signal compared to the signal from the three million cells.

Discussion

These results indicate that manganese does enter via the voltage-gated calcium channel, and manganese can be used as MRI contrast agent, which can also show the cell viability. Therefore, we suggest that manganese-guided MRI can be useful to monitor cell viability after the transplantation of human embryonic stem cells into the myocardium.

References

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