In vivo Molecular MRI of GFP/Ferritin Dual Gene Expression

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Introduction: Gene therapy holds great potential for treatment of various diseases. However, its clinical application was limited by lack of non-invasive imaging tools to monitor gene expression and therapy *in vivo*. MRI can provide high-resolution morphologic and functional imaging of tissues or organs, and therefore is one of the best imaging modalities for monitoring of gene expression and therapy by using a MR reporter gene [1, 2]. Currently, several MR imaging reporter genes have being developed for this purpose [3]. Among these genes, Ferritin, due to its working without use of other substrates and no cell toxicity, becomes the most attractive candidate as a MR reporter gene [4]. In this study, we aimed to develop a GFP and ferritin dual-gene vector for molecular MR imaging of gene expression, targeting non-invasive monitoring of gene expression or therapy *in vivo*.

Materials & methods: The murine ferritin heavy-chain DNA fragment (originally obtained from Weizmann Institute, Israel) was cut out and purified by gel electrophoresis. Then, the DNA fragment was inserted into a dual-gene expression vector, pBI-CMV2 plasmid, at BamHI and HindIII sites to simultaneously express GFP and ferritin in mammalian cells. Then, the plasmid was transfected into MCF7 cells. The expression of GFP was confirmed under a fluorescence microscope, and ferritin expression was confirmed by Western blot. For in *vivo* study, 25 μg pBI-GFP/mFerritin plasmid in 25μl buffer was injected in the left leg and the same volume of 5% sodium chloride solution was injected into the right leg of a mouse as the control, and then subjected to electroporation (8 square electrical pulses at 20ms pulse length and 100V/5mm field strength). Four days after electroporation, T2-weighted MRI was performed on a 3.0 T MRI scanner (Siemens, Erlangen, Germany) to visualize the ferritin gene expression in the legs. After MRI, the mice were sacrificed, and both sides of the legs were harvested for Prussian blue staining to confirm ferritin gene expression in the muscular skeleton cells.

Results and Discussion: The successful construction of the dual-gene plasmid was confirmed by digestion and gel electrophoresis (the data was not shown). The fluorescence microscopy imaging of transfected MCF7 cells showed the GFP expression in the cells (Fig.1). The Western blot analysis indicated the successful expression of ferritin in transfected MCF7 cells while the band is barely appeared in the untransfected cells (Fig.2). The T2-weighted MRI showed the ferritin gene expression (low signal intensity on T2MRI) in the left legs while no such findings in the right legs (Fig. 3), which was confirmed by the following Prussian blue staining (Fig. 4).

Conclusion: In this study, we successfully constructed pBI-GFP/mFerritin dual gene plasmid, which can simultaneously express GFP and ferritin confirmed by fluorescence microscope and Western blot *in vitro* and then T2-weighted MRI *in vivo*.

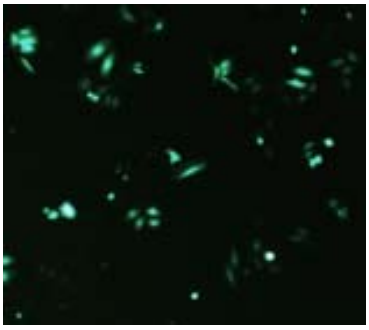

Fig.1 Fluorescence microscopy of pBI-GFP/mFerritin transfected MCF7 cells shows the successful GFP expression in the cells.

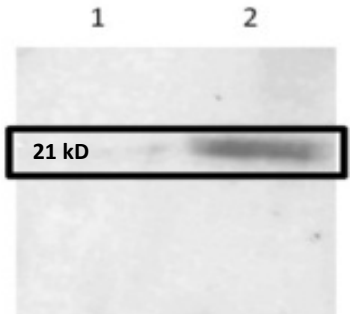

Fig.2 Western blot analysis indicates mferritin gene expression in the transfected cells and little background in the untransfected MCF7 cells.

Fig.3 Representative T2 weighted MRI of mouse legs shows low signal dots in the left leg while no such findings in the right leg.

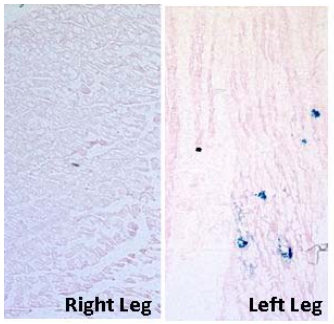

Fig.4. Prussian blue staining shows mFerritin expression / iron accumulation in the left leg while no such findings in the right leg.

References

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