MRI-based Generic Method for Imaging Transgene Expression

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Introduction

Transgenes, such as *LacZ*, are commonly used to report on gene expression *in vitro* by immunohistochemical methods. We have extended and adapted this methodology to provide a novel generic method to enable gene expression reporting by MRI and hence the possibility of imaging gene expression *in vivo*. The method, 'magnetic-antibody-gene imaging contrast' or *MAGIC*, combines MRI and the use of a surface antigen of choice to which a specific antibody recognises, the antibody being conjugated to an iron oxide particle (microbead), and hence MRI-visible. Also, in this study, we propose the use of a transgene coding for a modified form of the $H2K^k$ antigen, modified so as to prevent induction of intracellular cell signalling following antigen-antibody interaction. Previously, similar use of MRI and magnetic antibodies have been used to image gene expression of endogenous genes in specific cell types (1-4) but we have extended the methodology for use in the imaging of expression of transgenes or any gene of choice.

Methods and Materials

Cell transfection and labelling: HeLa cells were cultured under standard cell culture conditions and transfected with plasmids, pMACSKk or pMACS4.1 (Miltenyi Biotech Ltd). Forty-four hours later, the cells were harvested and then incubated with either anti-$H2K^k$ microbeads (MACSelect K^k microbeads, Miltenyi Biotech Ltd) or anti-$H2K^k$ FITC (Miltenyi Biotech Ltd), for MRI and electron microscopy (EM), or fluorescence activated cell sorting (FACS) and fluorescence microscopy, respectively.

MRI Data Collection and Analysis: MRI was performed on a 4.7T Inova MR scanner (Varian Inc., USA) of tubes containing cell pellets covered with excess PBS, placed in a water bath. To measure T2 values of the cell pellets, a spin-echo sequence was employed with varying TE (6 – 400 ms); TR, 3000 ms; FOV, 100x100 mm; matrix size, 256x128; 1 average and 1 slice (8 mm thick). Values of T2 were calculated by measuring the signal intensities (SI) of the cell pellets at various TE values using ImageJ (National Institute of Health, USA) and fitted to the equation: $SI = M_0 \exp^{(TE/T2)}$ using Prism (Graphpad Software Inc., USA).

Results and Discussions

Fig. 1 – FACS

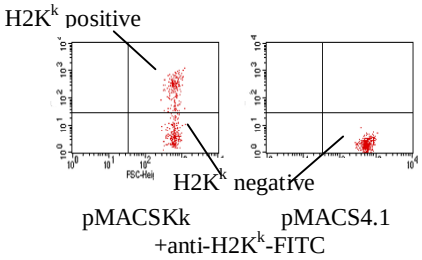


Fig. 2 – EM

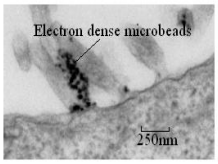
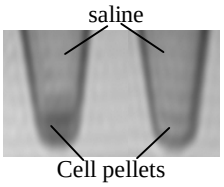


Fig. 3 - MRI



Expression of the $H2K^k$ antigen was confirmed by FACS, fluorescence and electron microscopy in only pMACSKk transfected cells (Figs. 1 and 2). HeLa cells transfected with either pMACSKk or pMACS4.1 (negative control) were incubated with MACSelect K^k microbeads for MRI. Strong negative contrast was observed in the MRI image of the former transfected cells compared to the latter at TE of 50 ms (Fig. 3). The T2 values of the pMACSKk and pMACS4.1 transfected cells were 57.6 ± 17.0 ms and 424.0 ± 38.7 ms, respectively ($P < 0.001$). Thus, the negative enhancement observed in the MRI image of the former cells is consistent with the significantly lower T2 values observed for the pMACSKk transfected cells. The EM showed the MACSelect K^k microbeads were present only on the surface of in the pMACSKk transfected cells (Fig. 2) and there was no evidence of internalisation of the microbeads. We have demonstrated the use of *MAGIC* for imaging transgene expression by MRI *in vitro*, using a modified form of the $H2K^k$ antigen. The *MAGIC* methodology can draw upon existing available immunohistochemical data to identify potential targets and antibodies, and a broad array of cell surface antigens, irrespective of their particular origin can be targeted. The use of iron oxide (magnetically) labelled antibodies renders *MAGIC* accessible to all researchers especially with the commercial availability of such entities for cell and macromolecular isolation by magnet technologies (5). This generic method has the advantage of being readily accessible to all researchers whilst having the flexibility to be tailored to individual researchers' needs.

Conclusion

We have shown the ability of *MAGIC* to image transgene expression *in vitro*. This methodology has great potential as a research tool and may provide a versatile platform for imaging novel gene targeted therapies *in vivo* or simply detecting gene expression in intact cells *in vitro*.

References

1. Kang *et* al., (2002), *Bioconj Chem*, 13, 122-127.
2. Weissleder *et al*., (1992), *Radiology*, 1992, 182(2), 381-5.
3. Pirko *et al*., (2004), *FASEB J 2004*, 18(1), 179-82.
4. Artemov *et al*., (2003), Mag Res Med, 49, 403-408.
5. Häfeli U, Schütt W, Teller J, Zborowski M, editors. In: Scientific and Clinical applications of magnetic carriers. New York: Plenum Press; 1997.

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