FOXP3 Gene Expression in Multiple Sclerosis patients before and after Mesenchymal Stem Cell therapy

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Maryam Mohajeri, Mandana Mohyeddin Bonab, Behrooz Nikbin, Ali Farazmand

1-Department of immunogenetic, School of Medicine, Tehran University of Medical Sciences
2-Department of Cell & Mol. Biology, School of Biology, Faculty of Science, University of Tehran

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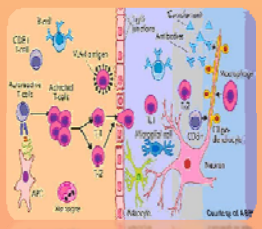
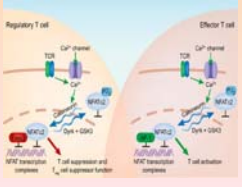
Introduction

Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disorder of the central nervous system (CNS). Activated T lymphocytes can cause inflammation by cytokine secretion in MS pathogenesis. In accordance to many observations, T lymphocytes can trigger the inflammation and neural injuries in MS pathogenesis and induce events, leading to the effector phase and axonal damage. Currently there is no successful treatment for MS, but one of the most capable neuroprotective strategies in research is the use of bone marrow-derived mesenchymal stem cells (MSC). These cells promote immune system regulation and possibly induction the neurological repair and re-myelinization of nerve cells. Recent studies show that MSC exert an immune regulatory function and induce T regulatory-cell proliferation, therefore can be a potentially useful treatment for autoimmune diseases including MS. In this pilot study a group of MS patients who underwented MSC therapy, assayed for expression of an X-linked transcription factor, *FOXP3*, as a specific marker of T Regulatory cells in peripheral blood. In most cases, all except one, qRT-PCR on PBMCs showed higher FoxP3 expression compared to the initial step, prior to injection.



FOXP3 Gene Expression

Functional features of forkhead transcription factor (FoxP3) has been found to be associated with CD4+ regulatory T cells (Fontenot JD. et al.,2003; Sakaguchi S. et al., 2005; Fontenot JD . et al., 2005).
FoxP3 seems predominantly be expressed in the CD4+CD25+regulatory T acells (T_{reg}), and ectopic expression of *FoxP3* in CD4+CD25+ T cells is sufficient to convert them into T_{reg} with strong suppressor activity (Khattri R. et al. 2003; Hori S. et al. 2003; Fontenot JD. et al., 2003). More notably, targeted mutation of *FoxP3* in hematopoietic stem cells is both necessary and sufficient to enable T_{reg} development (Sakaguchi S. et al., 2005). Thus, *FoxP3* considered being as a major regulator for the lineage differentiation and function of T_{reg} (Xing Chang. et al., 2006).
Recent studies in MS patients have shown the number of regulatory T cells; especially those expressing *FoxP3* may change during disease processes (Huan J. et al., 2005). To evaluate the effect of MSC on the T_{reg} in human, we examined the expression of *Foxp3* (as the marker of T_{reg} cells) in the peripheral blood mononuclear cells among MS patients before and after undergoing MSC therapy.



Material and Method

Bone marrow was taken from patients about one month prior to injection. Bone marrow MSCs were expanded in culture medium until to reach the desired number. The cells were harvested and prepared to injection. Then a mean volume of 10 ml containing at least $20X10^6$ cells were injected intrathecally to the patients. They were observed for 24 hours before being discharged from the hospital. Follow up of the patient condition was one year.
Peripheral blood mononuclear cells were obtained from 7 MS patients who received MSC. Samples were obtained in four interval times: one before injection of stem cells, and three have taken at one month, three months and finally six months following injection. Total RNA was extracted from PBMCs by TRYZOL (SIGMA) reagents, according to the manufacturer's protocol ().Agarose gel electrophoresis was done to check the RNA integrity (Figure 1). Then 1µg of this RNA converted to CDNA by fermentase reagents according to protocol (Fermentase, USA).

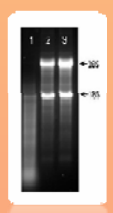
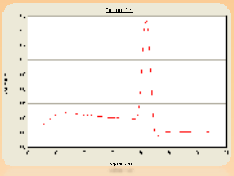


Figure 1. RNA integrity assesses.As shown in figure, one µg of total RNA was run on agarose gel and 28s And 18s bands were observed.

FOXP3 mRNA levels were quantified by real-time PCR with the ABI/PRISM 7500 sequence detection system (PE Applied Biosystems, Foster City, CA, USA). Real-time quantitative polymerase chain reaction (qPCR) was performed using SYBER GREEN I Gene Expression Assay for *FoxP3*. Relative expression was determined by normalization to *UBC* (Ubiquitin C) as a housekeeping gene.

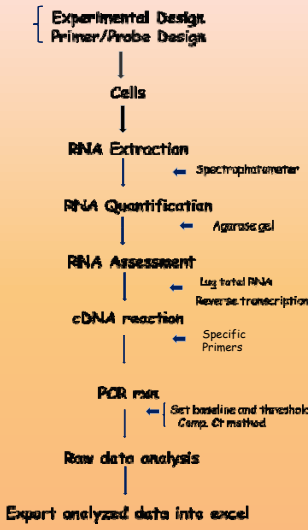
Melting curves of cDNAs were obtained to calibrate the threshold cycle to relative quantities of FOXP3 and UBC cDNAs in each sample (figure2).

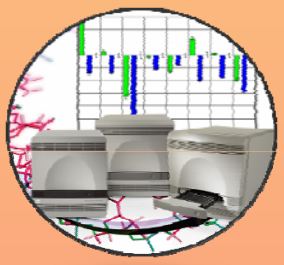


igure 2. Melting curve for FOXP3 cDNA. This unique pick of the curve indicates that the amplified sequence was specific.

Relative FOXP3 expression levels were calculated as [$2^{-\Delta\Delta Ct}$], Where $\Delta\Delta Ct = [\Delta Ct$ (sample) - ΔCt (calibrator)] and $\Delta Ct = [Ct$ (sample) –Ct (housekeeping)].
Statistical analysis was done with Non-parametric test, Wilcoxon & Freedman, using SPSS software

Steps of q RT-PCR





Results

All except one qRT-PCR on PBMCs showed *higher FoxP3 expression* compared to the initial step, before injection (Figure 3).

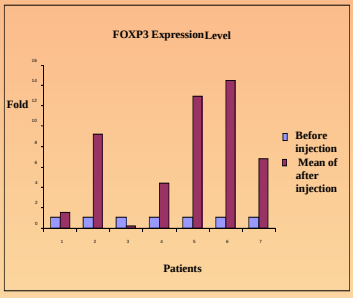


Figure 3. Quantitative analysis of *FoxP3* mRNA expressed in PBMCs driven from MS patients. Results in four stages indicate statistically significant differences in FOXP3 expression level.

For further information

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Conclusions

The in vivo results of the present study indicate that MSC may be effective in obtaining a sufficient number of T_{reg}, especially in the profusion of CD4/CD25 fraction for clinical purposes in MS patients because MSC preserves the T_{reg} function over time. These findings support former studies that employed MSC, through inducing T_{reg} cells, can ameliorate the symptoms of autoimmune diseases such as MS. Therefore, we may conclude that one of the effective mechanisms of MSC's function in treatment of autoimmunity is to induce and up regulate T_{reg} in human. As there are limited experiments on this issue, it seems that further studies using MSC may be necessary, if possible in patients at an earlier phase of the disease, to allow possible neurological regeneration prior to permanent changes occur in the CNS. Furthermore, culturing of bone marrow-derived MSC, possibly supplemented with biological agents, as cytokines can be beneficial to augment neurogenesis or to refresh degenerating neurons.

Acknowledgments

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