

Messenger RNA electroporation: an efficient tool in immunotherapy and stem cell research

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Abstract: Over the last decades medicine has developed tremendously, but still many diseases are incurable. The last years, cellular (gene) therapy has become a hot topic in biomedical research for the potential treatment of cancer, AIDS and diseases involving cell loss or degeneration. Here, we will focus on two major areas within cellular therapy, cellular immunotherapy and stem cell therapy, that could benefit from the introduction of neo-expressed genes through mRNA electroporation for basic research as well as for clinical applications. For cellular immunotherapy, we will provide a state-of-the-art on loading antigen-presenting cells with antigens in the mRNA format for manipulation of T cell immunity. In the area of stem cell research, we will highlight current gene transfer methods into adult and embryonic stem cells and discuss the use of mRNA electroporation for controlling guided differentiation of stem cells into specialized cell lineages.

Key words: mRNA - Electroporation - Immunotherapy - Stem cells - Antigen presenting cells - Dendritic cells

Introduction

The knowledge of medicine, immunology and biotechnology has progressed enormously, but still many diseases are incurable and have a large impact on the quality of life. Therefore many scientists are looking for new treatments for several diseases, like cancer, auto-immune diseases, pandemic infections (*e.g.* HIV), neurodegenerative diseases, *etc.* Several different approaches are studied, *e.g.* new sorts of drugs and combinations of radiotherapy and chemotherapy, but during the last years much attention has been paid to custom-made cellular therapies. In this review we will focus on the application of cellular therapy and mRNA electroporation in different cell types for the potential treatment of various diseases.

Use of mRNA electroporation for immunotherapy

The concept of immunotherapy in cancer and infectious diseases is based on the body's natural defence system that protects us against a variety of diseases and is a therapy that attempts to modify or enhance immune responses. The most studied cell types for immunotherapy are antigen-

presenting cells (APC) and T cells, which are pivotal players in initiating immune responses. Different strategies have been developed for loading APC with tumor or viral antigens [37]. Loading of characterized antigenic peptides is the most straightforward way, however disadvantages are the prior knowledge of the peptide epitopes, the short half-life of the human leukocyte antigen (HLA)/peptide complexes and the dependence on screening for appropriate HLA haplotypes in individual patients. Alternatively, viral and non-viral gene transfer technologies can be used. Recombinant viral vectors are characterized by a high transduction efficiency, but pose biosafety risks for clinical application; while transduction efficiency with viral vectors is high [10], plasmid DNA transfer into dendritic cells (DC), despite high clinical potential, has not been very efficient [34]. Nonviral non-DNA based gene delivery has several advantages, as compared to plasmid DNA and viral vectors, since there is no danger of insertional mutagenesis, no viral antigens and absence of vector-induced immunogenicity. The first report on the use of mRNA to load APC has come from the group of E. Gilboa [2,4,18]. The group of Gilboa applied passive RNA pulsing or RNA lipofection to introduce the coding RNA

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into DC. Our group was the first to describe an optimized electroporation protocol for the introduction of RNA into DC providing both biochemical evidence of transfection in terms of transgene EGFP expression as well as evidence of major histocompatibility complex (MHC) class I presentation [33]. Moreover, we demonstrated that mRNA electroporation of human DC was more efficient than plasmid DNA electroporation, and lipofection or passive pulsing of RNA for stimulation of antigen-specific CD8⁺ T cells, as confirmed by several other groups [11, 15, 27].

Dendritic cells

Thus far, most reports on mRNA electroporation have used dendritic cells (DC). As 'professional' antigen-capturing cells in an immature state and antigen-presenting cells after maturation, they play an important role in the activation of innate and adaptive immunity to pathogens, as well as in the maintenance of peripheral tolerance [30]. Especially the expression of costimulatory molecules on DC provides an extra immune stimulating signal to initiate a more efficient immune response. DC can be cultured from peripheral blood monocytes and therefore are readily accessible for use in cellular therapy. Antigen processing and subsequent MHC class I presentation on DC membrane after mRNA electroporation could be demonstrated using a CD8⁺ T cell clone for MAGE-A1 [24], Melan A [33] and WT1 (unpublished results). Transfection of mRNA encoding influenza (flu) matrix protein M1 allows loading of monocytes or DC for induction of autologous flu-specific CD8⁺ T cell activation [20, 22, 25]. After cryopreservation, mRNA-electroporated DC retained transgene expression, phenotypic properties and, most importantly, stimulatory capacity [25]. Recently, more reports on *in vitro* activation using RNA-electroporated dendritic cells have been published, generally regarding cancer-related antigens, *e.g.* telomerase [27], Melan A [11], total myeloma cell RNA [17]. Recently, we have observed that electroporation of HIV-1 *gag* mRNA can activate memory T cells from HIV patients *ex vivo* (unpublished results). Patients' autologous proviral DNA was PCR-amplified and DC electroporated with *in vitro* transcribed proviral *gag* mRNA stimulated autologous T cells. These findings open a major perspective for the development of patient-specific immunotherapy directed against the entire latent HIV quasispecies.

The loading of cells with mRNA is not only useful to transfect antigens, but can also be used to introduce costimulatory molecules in dendritic cells, like toll-like receptor (TLR) 4 [1, 5] and OX40L [7].

The use of mRNA-loaded DC has been very successful in *in vitro* experiments and now this strategy has been translated into several clinical trials. Cytotoxic T lymphocyte (CTL) responses were obtained in patients with

metastatic prostate tumors after vaccination with DC passively pulsed with prostate-specific antigen mRNA [9]. Nair *et al.* showed that vaccination with DC transfected with total tumor-derived mRNA stimulated a tumor-specific immune response in a patient with a carcino-embryonic antigen (CEA)-expressing adenocarcinoma [19]. Other clinical trials using RNA-loaded DC are still ongoing. We are currently recruiting acute myeloid leukemia (AML) patients in remission for a vaccine trial using WT1 mRNA-electroporated DC.

CD40-activated B cells

B cells are an alternative for DC and can be obtained from small quantities of peripheral blood. In contrast to the culture of DC starting from monocytes, B cells can proliferate and massive amounts can be cultured *in vitro* by CD40 activation, which makes them a cost-effective alternative for DC [28]. CD40-activated (CD40-) B cells have been shown in several *in vitro* systems to activate CTL responses against viral and tumor antigen targets, including neoantigens, that are formulated as peptides, proteins, or viral vectors [3, 12, 14]. Coughlin *et al.* reported that CD40-B cells transfected with RNA may serve as an alternative vaccine that can be generated from small blood volumes regardless of patient age [6]. They cultured CD40-B cells from pediatric patients with neuroblastoma and elicited cytotoxic T cells against several neuroblastoma-related antigens using tumor RNA-electroporated CD40-B cells. CD40-B cells can also be used to elicit CTL responses against infectious agents as demonstrated by Van den Bosch *et al.* for cytomegalovirus and influenza [31].

T cells

In the field of adoptive T cell immunotherapy, mRNA-based gene transfer into T cells could prove useful. Our group demonstrated that mRNA electroporation is feasible in activated T cells. However, in unstimulated T cells, mRNA electroporation turned out to be relatively inefficient [29].

Use of mRNA electroporation in stem cell research

Stem cell research, both embryonic and adult, offers several prospects towards the development of future cell-based therapies in regenerative medicine. The hope exists that (stem) cell transplantation will become part of an effective therapy for neurological disease or injury, diabetes and myocardial infarction. Although various combinations of growth factors and chemicals have been described for *in vitro* differentiation of stem cells into specific cell types, novel research is now focusing on the development of gene-based strategies in order to control and/or direct *in vivo* differentiation of transplanted stem

cell populations. For this, viral transduction techniques have been described as highly efficient for genetic modification of adult and embryonic stem cell populations [8, 16, 35], while currently optimized non-viral plasmid DNA-based transfection methods do not result in exceptionally high gene transfer efficiencies [13, 36]. As described before, one should consider the use of non-viral gene transfer methods when looking forward to clinical applications. In this context, we have investigated and introduced potential applications of mRNA-based gene transfer in stem cell research.

RNA-based gene transfer for adult and embryonic stem cells

For adult stem cell populations, we have shown that mRNA electroporation is a highly efficient gene transfer technology for *in vitro* cultured human bone marrow stromal cells (90% of gene transfer efficiency) and can also be used, although less efficiently at this time, for genetic loading of human CD34+ hematopoietic stem cells (35% of gene transfer efficiency) [29]. For embryonic stem cell populations, we demonstrated highly efficient cytoplasmic gene expression in 80-90% of mouse and human embryonic carcinoma (EC) stem cells (unpublished data) and mouse and human embryonic stem (ES) cells after electroporation with *in vitro* transcribed mRNA [21, 23, 26]. Moreover, next to the possibility of short term gene transfer via direct mRNA electroporation, electroporation with mRNA encoding Cre- or FLPe-recombinase proteins provides an easy and highly efficient method to induce sustained transgene expression in whole ES cell populations stably transfected with LoxP and/or FRT-flanked target sequences [21, 32].

Directing stem cell differentiation via RNA-based gene transfer

One of the research lines in our laboratory aims to investigate whether directed neural differentiation of embryonic cells can be induced after electroporation with mRNA encoding neural transcription factors and/or growth factors. In preliminary experiments, mouse P19 EC stem cells were electroporated with mRNA encoding various neurotrophic factors. Next, mRNA-electroporated P19 EC stem cells were allowed to form embryoid bodies during 4 days followed by flow cytometric analysis for the presence of neural progenitor cells based on A2B5 staining. While mock electroporation or electroporation with mRNA encoding EGFP, brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) did not result in directed neural differentiation, electroporation with mRNA encoding neurotrophin-3 (NT3) or electroporation with a mixture of BDNF, GDNF and NT3 mRNA resulted in an increased number of A2B5+ neural progenitor cells within cultured embryoid

bodies (unpublished data). These encouraging results on P19 EC stem cells provide proof-of-principle for the further development of RNA-based gene transfer strategies for triggering stem cell differentiation.

Conclusions and future perspectives

As outlined above, mRNA electroporation is a versatile powerful tool for obtaining short-term transgene expression in a variety of cell types. The use of mRNA-loaded DC has been very successful for *in vitro* purposes and has been recently translated into several clinical trials. The first results are very encouraging, as tumor specific immune responses were elicited [9, 19]. Several other clinical trials using RNA-loaded DC are still ongoing and will hopefully pave the way for improved DC therapy of cancer, but also for infectious diseases.

Messenger RNA electroporation is a promising method for genetic loading of stem cells with genes in order to direct their differentiation towards specialized cell types, which could be of use in the treatment of various diseases. However, clinical applicability can only be established after intensive laboratory studies, both *in vitro* and *in vivo*, that should elaborate on the safety and effectiveness of stem cell-based therapies.

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