European Journal of Pharmaceutics and Biopharmaceutics 71 (2009) 484–489

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Review article

Current prospects for mRNA gene delivery

Ayako Yamamoto [a,1], Michael Kormann [a,1], Joseph Rosenecker [a], Carsten Rudolph [a,b,*]

[a] Department of Pediatrics, Ludwig-Maximilians, University, Munich, Germany
[b] Department of Pharmacy, Free University of Berlin, Berlin, Germany

ARTICLE INFO

Article history:
Received 13 November 2007
Accepted in revised form 2 September 2008
Available online 10 October 2008

Keywords:
Gene therapy
Gene delivery
mRNA

ABSTRACT

Replication-deficient viruses have been used most successfully in the field of gene therapy because of their high transfection efficiency. However, the risk of insertional mutagenesis and induction of unwanted immune responses remains still critical for their safe application. On the other hand, nonviral vectors have been intensively investigated for plasmid DNA (pDNA) delivery as a safer alternative although their gene transfer efficiency is still many folds lower than for viral vectors, which has been predominately attributed to the insufficient transport of pDNA into the nucleus. Instead of pDNA, messenger RNA (mRNA) has recently emerged as an attractive and promising alternative in the nonviral gene delivery field. This strategy combines several advantages compared to pDNA: (i) the nuclear membrane, which is a major obstacle for pDNA, can be avoided because mRNA exerts its function in the cytoplasm; (ii) the risk of insertional mutagenesis can be excluded; (iii) the determination and use of an efficient promoter is omitted; (iv) repeated application is possible; (v) mRNA is also effective in non-dividing cells, and (vi) vector-induced immunogenicity may be avoidable. In this review, we summarize recent improvements of mRNA gene delivery and discuss its opportunities for the usage in gene therapy.

1. Introduction

Gene transfer vehicles based on mRNA have emerged as attractive alternatives to vehicles made of DNA for the potential treatment of genetic disorders or (anti-tumor) vaccination [1–4]. Its successful application has been demonstrated in cancer immunotherapy [5,6], not only because it is possible to deliver all epitopes of entire antigens in one step together but manipulation as well as purification are rather simple, too. In addition, this strategy has several advantages in terms of pharmaceutical safety because mRNA does not integrate into the genome and the transfection remains transient. mRNA encoding versatile antigens combined with delivery to dendritic cells (DCs) is a strong and promising approach to induce immune response in cancer patients [7,8].

Thus far, plasmid DNA (pDNA) has been largely used for nonviral gene transfer. However, it can hardly be transfected into non–dividing mammalian cells and, besides, bacterial unmethylated DNA CpG motifs induce strong immune response through Toll-like receptor 9 (TLR9) [9]. For example, only 1–10% of DCs are transfected by means of electroporation, cationic polymers or cationic lipids [10–13]. On the other hand, transfection efficiencies by electroporation of mRNA has been previously shown to reach up to 95% transfected cells [8]. These observations suggest that mRNA transfer is much more effective compared to pDNA transfer, for the most part because mRNA does not have to be transported into the nucleus. Consequently, early and dramatically higher protein expression has been reported [14–16].

Though the above listed advantages for the usage of mRNA for nonviral gene transfer should be emphasized, it must be noted that mRNA undergoes approximately 13 different nucleoside modifications including methylation in eukaryotic cells [17], and *in vitro* transcribed mRNA causes strong immune responses mediated by TLR3, TLR7 and TLR8 [18,19], which represents a major challenge for its successful *in vivo* application. However, modified nucleosides may contribute to a reduction of these immune stimulatory effects, as shall be discussed later [20].

2. Advances of *in vitro* transcription techniques

Mature mRNA in eukaryotic cells consists of five significant portions: the cap structure ([m^7Gp_3N (N: any nucleotide)], the 5′ untranslated region (5′UTR), an open reading frame (ORF), the 3′untranslated region (3′UTR), and a tail of 100–250 adenosine residues (Poly(A) tail) [21,22] (Fig. 1). *In vitro* transcribed mRNA can be obtained from plasmid DNA harboring a bacteriophage promoter, such as T7, SP6, or T3. *In vitro* transcription is a common technique using commercially available kits to obtain sufficient amounts of functional mRNA. Thus far, feasibility and technical refinement have been dramatically improved.

* Corresponding author. Division of Molecular Pulmonology, Department of Pediatrics, Ludwig-Maximilians, University, Lindwurmstrasse 2A, 80337 Munich, Germany. Tel.: +49 89 5160 7711; fax: +49 89 5160 4421.
E-mail address: Carsten.Rudolph@med.uni-muenchen.de (C. Rudolph).
[1] Both authors contributed equally to the paper.

0939-6411/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2008.09.016

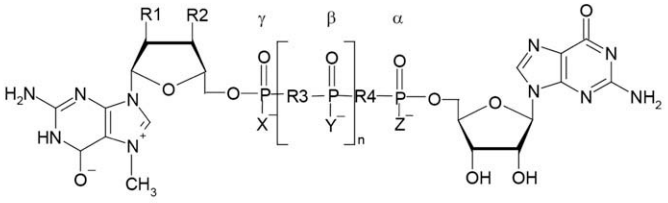
Cap	5‘ UTR	ORF	3‘ UTR	poly(A) tail

Fig. 1. Structure of eucaryotic mature mRNA; ORF, open reading frame; UTR, untranslated region.

It was found that one-third to one-half of the caps are incorporated in the reverse orientation during *in vitro* transcription [23], making them unrecognizable to the cap-binding protein, eukaryotic initiation factor 4E (eIF4E) [24–26]. Instead of the normal cap structure, Stepinski et al. discovered that an anti-reverse cap analog (ARCA), $m_2^{7,3'O}Gp_3G$ and $m^7 3'dGp_3G$ in which a 3′OH group of a normal cap is removed or replaced with OCH_3 could avoid the cap incorporation in the wrong orientation [27]. Subsequently, a high number of modifications on ARCA have been reported (Fig. 2). Intriguingly, Jemielity et al. found that modifications not only at the C3′ position, but also at the C2′ position prevent reverse incorporation [28]. Moreover, tetraphosphate ARCAs can promote translation more efficiently than other cap analogs [28]. As a result, *in vitro* ARCA-capped transcripts (ARCA-mRNA) showed significantly higher translation efficiency compared to normal capped transcripts (CAP-mRNA) in rabbit reticulocyte lysate [14]. Further, it has been reported that $m_2^{7,3'O}Gpp_{CH2}pG$ or $m_2^{7,3'O}Gp_{CH2}ppG$, in which the bridging oxygen in the α-β-linkage or β-γ-linkage, was substituted by a methylene group, respectively [29], were found to be resistant to hydrolysis by human Dcp2, one of the decapping enzymes, *in vitro*, and increased mRNA stability [30]. However, $m_2^{7,3'O}Gpp_{CH2}pG$ showed only 52–68% affinity for eIF4E compared to $m_2^{7,3'O}Gp_3G$ [30]. Grudzien-Nogalska et al. have recently reported that a phosphorothioate on ARCA (S-ARCA) stabilized and increased the efficiency of translation [31]. They found that luciferase (luc) mRNA capped with a sulfur substitution for a nonbridging oxygen in the β-phosphate moiety on ARCA, $m_2^{7,2'O}Gpp_spG$ (D2), was translated 5.1-fold more efficiently than a normal cap. Another diastereoisomeric form (D1) showed 2.8-fold higher translation efficiency [31]. There was no significant difference in the efficiency of translation between S-ARCA and ARCA. However, $t_{1/2}$ in D2 (257 min) was found to be strongly prolonged compared to normal cap (86 min) or ARCA (155 min) [31]. It seems therefore that the phosphorothioate contributes to the resistance to hydrolysis.

The poly(A) tail plays an important role in both mRNA translation and stability. The poly(A) tail binds to polyadenosyl binding protein (PABP) [32]. PABP interacts with the N-terminus of eIF4G, which leads to mRNA circularization [32]. In addition, the poly(A) tail is able to bind numerous PABPs, whose interaction with eIF4G results in an increase for the affinity of eIF4E to the cap structure. The Cap-poly(A) interaction cooperatively results from the physical interactions between mRNA 5′ and 3′ ends [33]. Once the poly(A) tail is removed or shortened to less than 12 residues, degradation of mRNA occurs through the cleavage of the 5′ cap structure and 5′ to 3′ exonucleotidic digestion or 3′ to 5′ degradation [34]. These observations illustrate that the poly(A) tail is very important to inhibit decapping as well as degradation of mRNA. For *in vitro* transcription, unless the template plasmid DNA contains a poly(d(A/T)) tail, it may be post-polyadenylated by the poly(A) polymerase. However, in this case the length of the poly(A) tail may vary from reaction to reaction and within one approach, although this variation remains surprisingly low (unpublished results).

Intriguingly, it has been reported that although capped polyadenylated mRNA translation was inhibited by the addition of exogenous poly(A) [35,36] *in trans*, the translation of capped non-polyadenylated mRNA was rather stimulated under certain poly(A) concentrations [37]. However, according to the report by Borman et al., the addition of exogenous poly(A) which consists of 10–180 residues *in trans* in rabbit reticulocyte lysates stimulated translation of capped mRNA with a 100 adenosine residue poly(A) tail 11-fold [38]. Mockey et al reported that the addition of a



Cap analog	R1	R2	R3	R4	X	Y	Z	n	relative translational efficiency	reference
m^7Gp_3G	OH	OH	O	O	O	O	O	1	1.00	24, 26, 27
$m^7 2'dGp_3G$	H	OH	O	O	O	O	O	1	1.17 ± 0.13	24
$m_2^{7,2'-O}Gp_3G$	OCH_3	OH	O	O	O	O	O	1	2.10 ± 0.2	27
$m^7 3'dGp_3G$	OH	H	O	O	O	O	O	1	2.13 ± 0.5	23
$m_2^{7,3'-O}Gp_3G$	OH	OCH_3	O	O	O	O	O	1	1.88 ± 0.40	24
$m_2^{7,3'-O}Gpp_{CH2}pG$	OH	OCH_3	O	CH_2	O	O	O	1	1.3 ± 0.1	26
$m_2^{7,3'-O}Gp_{CH2}ppG$	OH	OCH_3	CH_2	O	O	O	O	1	1.2 ± 0.2	26
$m_2^{7,2'-O}Gppp_sG$ (D1)	OCH_3	OH	O	O	O	O	S	1	2.5 ± 0.8	27
$m_2^{7,2'-O}Gppp_sG$ (D2)	OCH_3	OH	O	O	O	O	S	1	1.8 ± 0.4	27
$m_2^{7,2'-O}Gpp_spG$ (D1)	OCH_3	OH	O	O	O	S	O	1	2.8 ± 0.3	27
$m_2^{7,2'-O}Gpp_spG$ (D2)	OCH_3	OH	O	O	O	S	O	1	5.1 ± 0.5	27
$m_2^{7,2'-O}Gp_sppG$ (D1)	OCH_3	OH	O	O	S	O	O	1	2.0 ± 0.1	27
$m_2^{7,2'-O}Gp_sppG$ (D2)	OCH_3	OH	O	O	S	O	O	1	1.9 ± 0.1	27
$m_2^{7,3'-O}Gp_4G$	OH	OCH_3	O	O	O	O	O	2	2.42 ± 0.22	24
$m_2^{7,2'-O}Gp_4G$	OCH_3	OH	O	O	O	O	O	2	2.56 ± 0.18	24
$m^7 2'dGp_4G$	H	OH	O	O	O	O	O	2	1.83 ± 0.18	24
m^7Gp_5G	OH	OH	O	O	O	O	O	3	1.05 ± 0.08	24
$m_2^{7,3'-O}Gp_5G$	OH	OCH_3	O	O	O	O	O	3	1.39 ± 0.16	24

Fig. 2. Modifications of ARCA structures.

poly(A) tail in the range of 15–600 residues resulted in a 2.3-fold stimulation of protein expression by co-transfection of ARCA-luc mRNA-A100 using lipofection [14].

Besides, both the cap structure and the poly(A) tail have been reported to individually contribute to the level of protein expression. Mockey et al. found that ARCA-luc mRNA-A64 or 100 showed 25-fold, and 50-fold higher luciferase activity than CAP-luc mRNA-A64 or 100, respectively, using lipofection in mouse dendritic cells (JAWSII). In addition, ARCA-luc mRNA-A100 showed 700-fold higher luciferase activity than CAP-luc mRNA-A64. Hence, a long poly(A) tail combined with a modified cap structure, ARCA, greatly improves expression efficiency in dendritic cells [14].

It lies in the nature of enzymatic reactions that luciferase activity only indirectly measures protein expression levels, which means that the actual effects on translation efficiency remain to be determined. Elango et al. examined whether the length of the poly(A) tail (A0, A20, A40, A60, A80 and A100) affects expression levels not only in dendritic cells but also in other cells types. Interestingly, they found that the translation efficiency increased using a poly(A) tail with a length up to A60, then declined with increasing poly(A) tail length in UMR-106, an osteoblast-like osteosarcoma cell line from rat [16]. The effect of length of poly(A) on translation, therefore, might be cell type-dependent.

An important study was performed by Holtkamp et al. who intensively investigated the impact of mRNA modifications on its stability and translational efficiency in dendritic cells. In this study they discovered various important factors to increase the stability and translational efficiency of mRNA by (i) extending the length of poly(A) to A120; (ii) the use of type IIS restriction enzymes such as *SapI* and *BpiI* to avoid an overhang at the 3′ end of the poly(A) tail and to obtain a free-ending poly(A) tail when performing the linearization of the template plasmid vector; iii) two sequential 3′UTRs of the human β-globin gene cloned in between ORF and the poly(A) tail [15].

3. mRNA gene delivery systems

3.1. Nonviral gene transfer

A variety of transfection reagents have been evaluated for their ability to deliver mRNA. While until now most publications suggest lipoplexes for mRNA transfection, polyplexes based on polyethylenimine (PEI, 25 and 22 kDa) led to rather poor results. The use of polycations however has been only rarely described in literature, though DEAE-dextran [39], poly(L-lysine) [40] and dendrimers [13] were capable of transfecting mRNA into cells *in vitro*.

The feasibility of mRNA transfer in mammalian cells utilizing cationic lipids has been already described in the late 1980's [39]. DOTMA, a synthetic cationic lipid, incorporated into a liposome (lipofectin) was used to efficiently transfect mRNA into different cell lines *in vitro*. Different amounts of applied mRNA yielded a linear response of luciferase activity. Currently, DOTAP seems to be the most efficient and the most widely used cationic lipid, relatively cheap and efficient in both *in vitro* and *in vivo* mRNA delivery applications [41–43]. In addition, cationic polymers may be used for mRNA transfection. Read et al. described that certain synthetic vectors based on reducible polycations exceeded the mRNA transfection efficiencies of the gold standard 25 kDa PEI by far [44]. However, if those modified vectors can be used directly for *in vivo* gene transfer remains to be investigated. A comprehensive study was performed by Bettinger et al. who addressed the underlying transfection mechanisms in more detail [42]. One of their key findings was that the binding strength between the cationic polymer or lipid represented one of the critical parameters which affected mRNA expression efficiency. Whereas cationic polymers such as branched PEI 25 kDa and linear PEI 22 kDa, which were effective for pDNA delivery and tightly bound to mRNA, did not result in detectable expression, low molecular weight PEI 2 kDa bound mRNA less efficiently but led to high expression levels in the presence of endosomolytic agents such as chloroquin or chemically linked melittin comparable to DOTAP. These observations demonstrate that single stranded mRNA binding to cationic polymers is stronger than pDNA binding. This has further been demonstrated by Huth et al. who suggested that cytosolic RNA is involved in pDNA release from the cationic polymer as a precondition for nuclear entry and transcription [45]. Therefore, the design of novel cationic polymers for mRNA delivery has to carefully address nucleic-acid binding strength and efficient cationic polymers used for pDNA delivery may not be suitable for mRNA delivery.

Apart from polymeric and liposomal vector systems, an interesting further option that became prominent during the last years is the use of electroporation. Protocols for the delivery of exogenous RNA have been developed, resulting in 50–90% transfection efficiency in human hematopoietic cells and human embryonic stem cells [46,47]. As the mRNA does not have to enter the nucleus, soft electrical pulses may be applied, reducing cell toxicity. Another advantage of electroporation might be that the RNA is shuttled directly into the cytosol, therefore possibly not being sensed by innate RNA receptors, which could surpass unwanted immune responses as discussed in the next chapter.

3.2. Viral gene transfer

Recombinant viruses are commonly used for gene transfer. Usually, those viruses have some or all of the viral genes replaced by the relevant therapeutic or marker genes. Many viruses carry DNA or RNA that will be copied into DNA after transfection and which have to enter the nucleus. However, some RNA viruses have the advantage that expression and replication is localized in the cytoplasm. Theses positive strand viruses are characterized by a genome that may be directly translated into viral and therapeutic proteins by host ribosomes. In this group, especially alphaviruses (e.g. Sindbis and Semlike Forst virus), picornaviruses and flavivirus (e.g. Kunjin virus) have been utilized for gene therapy approaches. Several alphavirus vectors have become important due to their capacity to express large amounts of foreign protein in a broad range of different hosts [48]. Common strategies are either to directly substitute structural genes with heterologous sequences or to paste the heterologous genes downstream of a subgenomic promoter in tandem with the viral structural genes. In vaccine approaches the Venezuelan encephalitis virus (VEE) became quite prominent: attenuated VEE vaccines seem to protect against viral challenges from a variety of possible intake routes [49]. However, it has been shown that alphaviruses induce severe cytopathic effects which limit their use in gene therapy, although strategies were employed to overcome this problem [50].

Furthermore, the Sendai virus (SeV) should be explicitly mentioned, as it offers several advantages. Sendai virus is a negative sense, ssRNA virus of the *Paramyxoviridae* family. They adsorb to neuraminic acid containing membrane components, which primarily results in uptake not via endocytosis, but via membrane fusion, thereby potentially circumventing (endosomal) TLR recognition. Recombinant SeV vectors (SeVV) are free from genotoxicity and are capable of infecting and multiplying in a wide variety of mammalian cells and tissues [51]. In contrast to positive strand genomes, negative sense RNA is not infectious when introduced into an appropriate host cell, as it must be coupled with its own RNA-dependent RNA polymerase to result in the positive sense RNA. Additionally, SeV is responsible for respiratory tract infection only in mice, hamsters, guinea pigs, rats, and occassionally pigs, but causes no disease in humans. First, SeVV has been suc-

cessfully engineered recombinantly to express enhanced green fluorescent protein (EGFP) [52], luciferase [53] and β-galactosidase [54]. This was followed by introducing genes coding for secretable reporter proteins like secreted alkaline phophatase [55] and secretable therapeutic proteins like human clotting factor IX [56], Interleukin-10 [57], and human Insulin-like growth factor-1 [58]. Recent developments refined SeV application, showing that IL-2 encoding SeVV induces significant regression of established rat brain tumors [59], SeVV gene delivery of Paraoxonase-1 inhibits neointimal hyperplasia in rabbits [60] and administration of cystic fibrosis transmembrane conductance regulator (CFTR) cDNA introduced into SeV led to partial correction of the CF chloride transport defect in CFTR mutant mice [61].

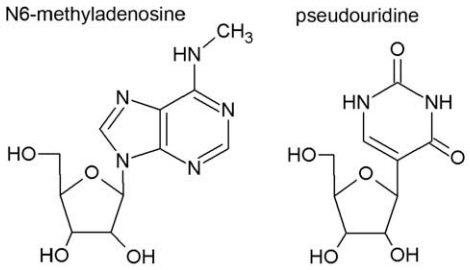


Fig. 3. Modifications of nucleosides.

4. Immune reactions in response to mRNA application

It has already been shown that *in vivo* application of bacterial DNA may lead to strong immune responses, especially via unmethylated CpG motifs [62–65]. In contrast to naked DNA, which induces only a mild cytokine response, its complexes with cationic lipids lead to a strong cytokine response [66–68]. Whereas varying the administration routes of cationic lipids did not markedly alter inflammatory cytokine expression, PEI-DNA either after intravenous injection or aerosol delivery resulted in lower lung cytokine levels compared to cationic lipids [69] presumably due to their different endosomal uptake and thus interaction with the TLR9 receptor [70].

Responses to RNA delivery are much less explored. Both DNA and RNA stimulate the mammalian innate immune system through activation of Toll-like receptors (TLRs). Thirteen TLRs (named simply TLR1 to TLR13) have been identified in humans and mice together, and equivalent forms of many of these have been found in other mammalian species [71–73]. Remarkably, different TLRs can recognize several structurally unrelated ligands [74–76]. The TLR-mediated innate immune system has a bow-tie architecture [77] in which a variety of pathogens and their molecules are represented by a much smaller number of ligands. The subcellular localization of different TLRs correlates to some extent with the molecular patterns of their ligands. Hence, TLR3, TLR7, TLR8 and TLR9 – all of which are involved in the recognition of nucleic-acid like structures, are localized intracellularly [78–80]. TLR3 recognizes dsRNA [81], siRNA [82] and mRNA [19], while TLR7 and TLR8 bind ssRNA [83] and the recognition of CpG DNA motifs is mediated via TLR9 [84,85].

In line with DNA CpG methylation (that suppresses recognition via TLR9), the immunogenicity of RNA seems to be under the control of similar types of modification. Karikó et al. found that *in vitro* transcribed RNA resulted in strong TNF-alpha response by dendritic cells if they showed no mammalian-typical modifications [20]. Intriguingly, the modification of specific nucleotides (e.g. N6-methyladenosine and pseudouridine, Fig. 3) reduced the TLR3, TLR7 and TLR8 mediated cytokine secretion and activation of DCs dramatically. Hence, it may be possible to eliminate exaggerated *in vivo* immune responses by introducing modified NTPs into the *in vitro* transcription reaction. However, it is currently unknown if those modifications have any impact on TLR-independent recognition via e.g. RIG-1 and MDA-5. The latter are cytosolic RNA helicases that detect viral RNA and trigger type I IFN through an IRF-3-dependent pathway [86]. Therefore, detailed *in vivo* studies – preferably in knock-out mice lacking certain TLRs or other pattern recognition receptors – with different transfection methods have to be performed to determine the real benefit of introducing altered nucleotides into mRNA.

On the other hand, the strong immunostimulatory effect of RNA is used for therapeutic vaccination. Especially dendritic cells (DCs) as antigen presenting cells (APCs) are targeted by vaccine immunogens, which is followed by an activation of antigen-specific T and B cells (recently reviewed in [87]). Several *in vivo* and *in vitro* studies have shown that targeting DCs with mRNA induced tumor immunity or anti-tumor responses [88–90]. Compared to transfection of pDNA, mRNA based gene transfer led to higher tumor antigen loading of DCs and had a higher potential to stimulate cytotoxic T lymphocyte responses [13,46]. Apart from anti-tumor approaches the ambition arised to use RNA-transfected DCs to cure or prevent infectious diseases like AIDS, hepatitis C or fungal infection [91–93]. Another elegant strategy to achieve RNA vaccination is to express a target antigen by a bi-cistronic replicative RNA which codes both for the antigen and a RNA replicase [94,95], thereby utilizing the ability of alphaviruses to produce large amounts of viral mRNA [96]. If a cell is transfected, the viral RNA is amplified by the replicase complex which synthesizes a genomic negative-strand that itself represents the template for the synthesis of many genomic RNA positive-strands by the RNA replicase. This approach has already been used in a mouse model to break tolerance and provide immunity to melanoma [97].

5. Future directions

The use of mRNA based gene transfer could indeed be a promising new strategy for the treatment of congenital and acquired diseases. No risk of integration into genomic DNA, strong and adjustable expression, and the fact that the transfection efficacy is independent of the cell cycle of target cells, are just some advantages, among others. Transfection protocols for mRNA are similar to well established plasmid DNA transfection and are, compared with viral transduction protocols, simple. Several papers have shown that mRNA can indeed be used to deliver and express transgenes in mammalian tissues. Recently Probst et al. could demonstrate that *in vivo* delivery of mRNA into human skin led to local gene expression [98]. However, especially for the potential usage of mRNA for the treatment of inherited diseases, the reduction of resulting inflammatory reactions represents a major challenge which has not been carefully addressed to date. By taming or utilizing the immunogenic properties, mRNA offers a promising tool for both gene therapy for inherited diseases and vaccination approaches, respectively. Although principally repeated mRNA application seems to be feasible, knowledge about application frequencies for the long-term treatment of inherited diseases are currently unknown. Thus, short-term expression of mRNA may be critical and could be one of the major limitations for mRNA application for the treatment of inherited diseases. However, long-term correction of genetic disorders at the genomic level could be achieved when mRNA encoding custom-made zinc-finger nucleases [99] to specifically induce double strand breaks was combined together with homologous sequence on a repair template DNA that locally stimulates homologous recombination,

which may further be applied to regenerative medicine together with stem cell technology.

Acknowledgments

This work was supported by the German Federeal Ministry of Education and Research in the program Innovative Medicines, Grants 01GU0616 TP4 and 01GU0616 TP1, and BioFuture (0311898).

References

[1] M. Mockey, E. Bourseau, V. Chandrashekhar, A. Chaudhuri, S. Lafosse, E. Le Cam, V.F. Quesniaux, B. Ryffel, C. Pichon, P. Midoux, mRNA-based cancer vaccine: prevention of B16 melanoma progression and metastasis by systemic injection of MART1 mRNA histidylated lipopolyplexes, Cancer Gene. Ther. 14 (2007) 802–814.
[2] R.M. Conry, A.F. LoBuglio, M. Wright, L. Sumerel, M.J. Pike, F. Johanning, R. Benjamin, D. Lu, D.T. Curiel, Characterization of a messenger RNA polynucleotide vaccine vector, Cancer Res. 55 (1995) 1397–1400.
[3] F. Martinon, S. Krishnan, G. Lenzen, R. Magne, E. Gomard, J.G. Guillet, J.P. Levy, P. Meulien, Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA, Eur. J. Immunol. 23 (1993) 1719–1722.
[4] D. Boczkowski, S.K. Nair, D. Snyder, E. Gilboa, Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo, J. Exp. Med. 184 (1996) 465–472.
[5] J.P. Carralot, J. Probst, I. Hoerr, B. Scheel, R. Teufel, G. Jung, H.G. Rammensee, S. Pascolo, Polarization of immunity induced by direct injection of naked sequence-stabilized mRNA vaccines, Cell. Mol. Life Sci. 61 (2004) 2418–2424.
[6] I. Hoerr, R. Obst, H.G. Rammensee, G. Jung, In vivo application of RNA leads to induction of specific cytotoxic T lymphocytes and antibodies, Eur. J. Immunol. 30 (2000) 1–7.
[7] A. Heiser, D. Coleman, J. Dannull, D. Yancey, M.A. Maurice, C.D. Lallas, P. Dahm, D. Niedzwiecki, E. Gilboa, J. Vieweg, Autologous dendritic cells transfected with prostate-specific antigen RNA stimulate CTL responses against metastatic prostate tumors, J. Clin. Invest. 109 (2002) 409–417.
[8] P. Ponsaerts, V.F. Van Tendeloo, Z.N. Berneman, Cancer immunotherapy using RNA-loaded dendritic cells, Clin. Exp. Immunol. 134 (2003) 378–384.
[9] A.M. Krieg, CpG motifs in bacterial DNA and their immune effects, Annu. Rev. Immunol. 20 (2002) 709–760.
[10] J.F. Arthur, L.H. Butterfield, M.D. Roth, L.A. Bui, S.M. Kiertscher, R. Lau, S. Dubinett, J. Glaspy, W.H. McBride, J.S. Economou, A comparison of gene transfer methods in human dendritic cells, Cancer Gene. Ther. 4 (1997) 17–25.
[11] S.S. Diebold, M. Kursa, E. Wagner, M. Cotten, M. Zenke, Mannose polyethylenimine conjugates for targeted DNA delivery into dendritic cells, J. Biol. Chem. 274 (1999) 19087–19094.
[12] S.S. Diebold, H. Lehrmann, M. Kursa, E. Wagner, M. Cotten, M. Zenke, Efficient gene delivery into human dendritic cells by adenovirus polyethylenimine and mannose polyethylenimine transfection, Hum. Gene. Ther. 10 (1999) 775–786.
[13] I. Strobel, S. Berchtold, A. Gotze, U. Schulze, G. Schuler, A. Steinkasserer, Human dendritic cells transfected with either RNA or DNA encoding influenza matrix protein M1 differ in their ability to stimulate cytotoxic T lymphocytes, Gene Ther. 7 (2000) 2028–2035.
[14] M. Mockey, C. Goncalves, F.P. Dupuy, F.M. Lemoine, C. Pichon, P. Midoux, mRNA transfection of dendritic cells: synergistic effect of ARCA mRNA capping with Poly(A) chains in cis and in trans for a high protein expression level, Biochem. Biophys. Res. Commun. 340 (2006) 1062–1068.
[15] S. Holtkamp, S. Kreiter, A. Selmi, P. Simon, M. Koslowski, C. Huber, O. Tureci, U. Sahin, Modification of antigen-encoding RNA increases stability, translational efficacy, and T-cell stimulatory capacity of dendritic cells, Blood 108 (2006) 4009–4017.
[16] N. Elango, S. Elango, P. Shivshankar, M.S. Katz, Optimized transfection of mRNA transcribed from a d(A/T)100 tail-containing vector, Biochem. Biophys. Res. Commun. 330 (2005) 958–966.
[17] J. Rozenski, P.F. Crain, J.A. McCloskey, The RNA Modification Database: 1999 update, Nucleic Acids Res. 27 (1999) 196–197.
[18] K.J. Ishii, S. Akira, TLR ignores methylated RNA?, Immunity 23 (2005) 111–113
[19] K. Kariko, H. Ni, J. Capodici, M. Lamphier, D. Weissman, mRNA is an endogenous ligand for Toll-like receptor 3, J. Biol. Chem. 279 (2004) 12542–12550.
[20] K. Kariko, M. Buckstein, H. Ni, D. Weissman, Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA, Immunity 23 (2005) 165–175.
[21] A.K. Banerjee, 5′-terminal cap structure in eucaryotic messenger ribonucleic acids, Microbiol. Rev. 44 (1980) 175–205.
[22] R.J. Jackson, Cytoplasmic regulation of mRNA function: the importance of the 3′ untranslated region, Cell 74 (1993) 9–14.
[23] A.E. Pasquinelli, J.E. Dahlberg, E. Lund, Reverse 5′ caps in RNAs made in vitro by phage RNA polymerases, RNA 1 (1995) 957–967.
[24] A. Cai, M. Jankowska-Anyszka, A. Centers, L. Chlebicka, J. Stepinski, R. Stolarski, E. Darzynkiewicz, R.E. Rhoads, Quantitative assessment of mRNA cap analogues as inhibitors of in vitro translation, Biochemistry 38 (1999) 8538–8547.
[25] J. Marcotrigiano, A.C. Gingras, N. Sonenberg, S.K. Burley, Cocrystal structure of the messenger RNA 5′ cap-binding protein (eIF4E) bound to 7-methyl-GDP, Cell 89 (1997) 951–961.
[26] H. Matsuo, H. Li, A.M. McGuire, C.M. Fletcher, A.C. Gingras, N. Sonenberg, G. Wagner, Structure of translation factor eIF4E bound to m7GDP and interaction with 4E-binding protein, Nat. Struct. Biol. 4 (1997) 717–724.
[27] J. Stepinski, C. Waddell, R. Stolarski, E. Darzynkiewicz, R.E. Rhoads, Synthesis and properties of mRNAs containing the novel "anti-reverse" cap analogs 7-methyl(3′-O-methyl)GpppG and 7-methyl (3′-deoxy)GpppG, RNA 7 (2001) 1486–1495.
[28] J. Jemielity, T. Fowler, J. Zuberek, J. Stepinski, M. Lewdorowicz, A. Niedzwiecka, R. Stolarski, E. Darzynkiewicz, R.E. Rhoads, Novel "anti-reverse" cap analogs with superior translational properties, RNA 9 (2003) 1108–1122.
[29] M. Kalek, J. Jemielity, Z.M. Darzynkiewicz, E. Bojarska, J. Stepinski, R. Stolarski, R.E. Davis, E. Darzynkiewicz, Enzymatically stable 5′ mRNA cap analogs: synthesis and binding studies with human DcpS decapping enzyme, Bioorg. Med. Chem. 14 (2006) 3223–3230.
[30] E. Grudzien, M. Kalek, J. Jemielity, E. Darzynkiewicz, R.E. Rhoads, Differential inhibition of mRNA degradation pathways by novel cap analogs, J. Biol. Chem. 281 (2006) 1857–1867.
[31] E. Grudzien-Nogalska, J. Jemielity, J. Kowalska, E. Darzynkiewicz, R.E. Rhoads, Phosphorothioate cap analogs stabilize mRNA and increase translational efficiency in mammalian cells, RNA 13 (2007) 1745–1755.
[32] D.R. Gallie, A tale of two termini: a functional interaction between the termini of an mRNA is a prerequisite for efficient translation initiation, Gene 216 (1998) 1–11.
[33] J. Coller, R. Parker, Eukaryotic mRNA decapping, Annu. Rev. Biochem. 73 (2004) 861–890.
[34] D. Schwartz, C.J. Decker, R. Parker, The enhancer of decapping proteins, Edc1p and Edc2p, bind RNA and stimulate the activity of the decapping enzyme, RNA 9 (2003) 239–251.
[35] D.R. Gallie, R. Tanguay, Poly(A) binds to initiation factors and increases cap-dependent translation in vitro, J. Biol. Chem. 269 (1994) 17166–17173.
[36] A. Jacobson, M. Favreau, Possible involvement of poly(A) in protein synthesis, Nucleic Acids Res. 11 (1983) 6353–6368.
[37] D. Munroe, A. Jacobson, mRNA poly(A) tail, a 3′ enhancer of translational initiation, Mol. Cell. Biol. 10 (1990) 3441–3455.
[38] A.M. Borman, Y.M. Michel, C.E. Malnou, K.M. Kean, Free poly(A) stimulates capped mRNA translation in vitro through the eIF4G-poly(A)-binding protein interaction, J. Biol. Chem. 277 (2002) 36818–36824.
[39] R.W. Malone, P.L. Felgner, I.M. Verma, Cationic liposome-mediated RNA transfection, Proc. Natl. Acad. Sci. USA 86 (1989) 6077–6081.
[40] K.J. Fisher, J.M. Wilson, The transmembrane domain of diphtheria toxin improves molecular conjugate gene transfer, Biochem. J. 321 (Pt. 1) (1997) 49–58.
[41] F.T. Zohra, E.H. Chowdhury, S. Tada, T. Hoshiba, T. Akaike, Effective delivery with enhanced translational activity synergistically accelerates mRNA-based transfection, Biochem. Biophys. Res. Commun. 358 (2007) 373–378.
[42] T. Bettinger, R.C. Carlisle, M.L. Read, M. Ogris, L.W. Seymour, Peptide-mediated RNA delivery: a novel approach for enhanced transfection of primary and post-mitotic cells, Nucleic Acids Res. 29 (2001) 3882–3891.
[43] D. Lu, R. Benjamin, M. Kim, R.M. Conry, D.T. Curiel, Optimization of methods to achieve mRNA-mediated transfection of tumor cells in vitro and in vivo employing cationic liposome vectors, Cancer Gene Ther. 1 (1994) 245–252.
[44] M.L. Read, S. Singh, Z. Ahmed, M. Stevenson, S.S. Briggs, D. Oupicky, L.B. Barrett, R. Spice, M. Kendall, M. Berry, J.A. Preece, A. Logan, L.W. Seymour, A versatile reducible polycation-based system for efficient delivery of a broad range of nucleic acids, Nucleic Acids Res. 33 (2005) e86.
[45] S. Huth, F. Hoffmann, K. von Gersdorff, A. Laner, D. Reinhardt, J. Rosenecker, C. Rudolph, Interaction of polyamine gene vectors with RNA leads to the dissociation of plasmid DNA-carrier complexes, J. Gene Med. 8 (2006) 1416–1424.
[46] V.F. Van Tendeloo, P. Ponsaerts, F. Lardon, G. Nijs, M. Lenjou, C. Van Broeckhoven, D.R. Van Bockstaele, Z.N. Berneman, Highly efficient gene delivery by mRNA electroporation in human hematopoietic cells: superiority to lipofection and passive pulsing of mRNA and to electroporation of plasmid cDNA for tumor antigen loading of dendritic cells, Blood 98 (2001) 49–56.
[47] P. Ponsaerts, S. van der Sar, V.F. Van Tendeloo, P.G. Jorens, Z.N. Berneman, P.B. Singh, Highly efficient mRNA-based gene transfer in feeder-free cultured H9 human embryonic stem cells, Cloning Stem Cells 6 (2004) 211–216.
[48] J.H. Strauss, E.G. Strauss, The alphaviruses: gene expression, replication, and evolution, Microbiol. Rev. 58 (1994) 491–562.
[49] P.C. Charles, K.W. Brown, N.L. Davis, M.K. Hart, R.E. Johnston, Mucosal immunity induced by parenteral immunization with a live attenuated Venezuelan equine encephalitis virus vaccine candidate, Virology 228 (1997) 153–160.
[50] E.V. Agapov, I. Frolov, B.D. Lindenbach, B.M. Pragai, S. Schlesinger, C.M. Rice, Noncytopathic Sindbis virus RNA vectors for heterologous gene expression, Proc. Natl. Acad. Sci. USA 95 (1998) 12989–12994.
[51] M. Bitzer, S. Armeanu, U.M. Lauer, W.J. Neubert, Sendai virus vectors as an emerging negative-strand RNA viral vector system, J. Gene Med. 5 (2003) 543–553.
[52] D.R. Agungpriyono, R. Yamaguchi, K. Uchida, Y. Tohya, A. Kato, Y. Nagai, M. Asakawa, S. Tateyama, Green fluorescent protein gene insertion of Sendai

Virus infection in nude mice: possibility as an infection tracer, J. Vet. Med. Sci. 62 (2000) 223–228.
[53] M.K. Hasan, A. Kato, T. Shioda, Y. Sakai, D. Yu, Y. Nagai, Creation of an infectious recombinant Sendai virus expressing the firefly luciferase gene from the 3′ proximal first locus, J. Gen. Virol. 78 (Pt. 11) (1997) 2813–2820.
[54] Y. Yonemitsu, C. Kitson, S. Ferrari, R. Farley, U. Griesenbach, D. Judd, R. Steel, P. Scheid, J. Zhu, P.K. Jeffery, A. Kato, M.K. Hasan, Y. Nagai, I. Masaki, M. Fukumura, M. Hasegawa, D.M. Geddes, E.W. Alton, Efficient gene transfer to airway epithelium using recombinant Sendai virus, Nat. Biotechnol. 18 (2000) 970–973.
[55] T. Tokusumi, A. Iida, T. Hirata, A. Kato, Y. Nagai, M. Hasegawa, Recombinant Sendai viruses expressing different levels of a foreign reporter gene, Virus Res. 86 (2002) 33–38.
[56] M. Bitzer, G. Ungerechts, S. Bossow, F. Graepler, R. Sedlmeier, S. Armeanu, C. Bernloehr, M. Spiegel, C.D. Gross, M. Gregor, W.J. Neubert, U.M. Lauer, Negative-strand RNA viral vectors: intravenous application of Sendai virus vectors for the systemic delivery of therapeutic genes, Mol. Ther. 7 (2003) 210–217.
[57] U. Griesenbach, R.L. Cassady, S. Ferrari, M. Fukumura, C. Muller, E. Schmitt, J. Zhu, P.K. Jeffery, Y. Nagai, D.M. Geddes, M. Hasegawa, E.W. Alton, The nasal epithelium as a factory for systemic protein delivery, Mol. Ther. 5 (2002) 98–103.
[58] A. Shiotani, M. Fukumura, M. Maeda, X. Hou, M. Inoue, T. Kanamori, S. Komaba, K. Washizawa, S. Fujikawa, T. Yamamoto, C. Kadono, K. Watabe, H. Fukuda, K. Saito, Y. Sakai, Y. Nagai, J. Kanzaki, M. Hasegawa, Skeletal muscle regeneration after insulin-like growth factor I gene transfer by recombinant Sendai virus vector, Gene Ther. 8 (2001) 1043–1050.
[59] Y. Iwadate, M. Inoue, T. Saegusa, Y. Tokusumi, H. Kinoh, M. Hasegawa, M. Tagawa, A. Yamaura, H. Shimada, Recombinant Sendai virus vector induces complete remission of established brain tumors through efficient interleukin-2 gene transfer in vaccinated rats, Clin. Cancer Res. 11 (2005) 3821–3827.
[60] M. Miyoshi, Y. Nakano, T. Sakaguchi, H. Ogi, N. Oda, K. Suenari, K. Kiyotani, R. Ozono, T. Oshima, T. Yoshida, K. Chayama, Gene delivery of paraoxonase-1 inhibits neointimal hyperplasia after arterial balloon-injury in rabbits fed a high-fat diet, Hypertens. Res. 30 (2007) 85–91.
[61] S. Ferrari, U. Griesenbach, A. Iida, R. Farley, A.M. Wright, J. Zhu, F.M. Munkonge, S.N. Smith, J. You, H. Ban, M. Inoue, M. Chan, C. Singh, B. Verdon, B.E. Argent, B. Wainwright, P.K. Jeffery, D.M. Geddes, D.J. Porteous, S.C. Hyde, M.A. Gray, M. Hasegawa, E.W. Alton, Sendai virus-mediated CFTR gene transfer to the airway epithelium, Gene Ther. 14 (2007) 1371–1379.
[62] Z.K. Ballas, W.L. Rasmussen, A.M. Krieg, Induction of NK activity in murine and human cells by CpG motifs in oligodeoxynucleotides and bacterial DNA, J. Immunol. 157 (1996) 1840–1845.
[63] M.D. Halpern, R.J. Kurlander, D.S. Pisetsky, Bacterial DNA induces murine interferon-gamma production by stimulation of interleukin-12 and tumor necrosis factor-alpha, Cell Immunol. 167 (1996) 72–78.
[64] A.M. Krieg, A.K. Yi, S. Matson, T.J. Waldschmidt, G.A. Bishop, R. Teasdale, G.A. Koretzky, D.M. Klinman, CpG motifs in bacterial DNA trigger direct B-cell activation, Nature 374 (1995) 546–549.
[65] Y. Tan, S. Li, B.R. Pitt, L. Huang, The inhibitory role of CpG immunostimulatory motifs in cationic lipid vector-mediated transgene expression in vivo, Hum. Gene Ther. 10 (1999) 2153–2161.
[66] B.D. Freimark, H.P. Blezinger, V.J. Florack, J.L. Nordstrom, S.D. Long, D.S. Deshpande, S. Nochumson, K.L. Petrak, Cationic lipids enhance cytokine and cell influx levels in the lung following administration of plasmid: cationic lipid complexes, J. Immunol. 160 (1998) 4580–4586.
[67] S. Li, S.P. Wu, M. Whitmore, E.J. Loeffert, L. Wang, S.C. Watkins, B.R. Pitt, L. Huang, Effect of immune response on gene transfer to the lung via systemic administration of cationic lipidic vectors, Am. J. Physiol. 276 (1999) L796–L804.
[68] N.S. Yew, H. Zhao, I.H. Wu, A. Song, J.D. Tousignant, M. Przybylska, S.H. Cheng, Reduced inflammatory response to plasmid DNA vectors by elimination and inhibition of immunostimulatory CpG motifs, Mol. Ther. 1 (2000) 255–262.
[69] A. Gautam, C.L. Densmore, J.C. Waldrep, Pulmonary cytokine responses associated with PEI-DNA aerosol gene therapy, Gene Ther. 8 (2001) 254–257.
[70] S. Kawakami, Y. Ito, P. Charoensit, F. Yamashita, M. Hashida, Evaluation of proinflammatory cytokine production induced by linear and branched polyethylenimine/plasmid DNA complexes in mice, J. Pharmacol. Exp. Ther. 317 (2006) 1382–1390.
[71] T.H. Chuang, R.J. Ulevitch, Cloning and characterization of a sub-family of human toll-like receptors: hTLR7, hTLR8 and hTLR9, Eur. Cytokine Netw. 11 (2000) 372–378.
[72] X. Du, A. Poltorak, Y. Wei, B. Beutler, Three novel mammalian toll-like receptors: gene structure, expression, and evolution, Eur. Cytokine Netw. 11 (2000) 362–371.
[73] K. Tabeta, P. Georgel, E. Janssen, X. Du, K. Hoebe, K. Crozat, S. Mudd, L. Shamel, S. Sovath, J. Goode, L. Alexopoulou, R.A. Flavell, B. Beutler, Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection, Proc. Natl. Acad. Sci. USA 101 (2004) 3516–3521.
[74] S. Akira, K. Takeda, T. Kaisho, Toll-like receptors: critical proteins linking innate and acquired immunity, Nat. Immunol. 2 (2001) 675–680.
[75] C.A. Janeway Jr., R. Medzhitov, Innate immune recognition, Annu. Rev. Immunol. 20 (2002) 197–216.
[76] R. Medzhitov, Toll-like receptors and innate immunity, Nat. Rev. Immunol. 1 (2001) 135–145.
[77] M. Csete, J. Doyle, Bow ties, metabolism and disease, Trends Biotechnol. 22 (2004) 446–450.
[78] P. Ahmad-Nejad, H. Hacker, M. Rutz, S. Bauer, R.M. Vabulas, H. Wagner, Bacterial CpG-DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments, Eur. J. Immunol. 32 (2002) 1958–1968.
[79] F. Heil, P. Ahmad-Nejad, H. Hemmi, H. Hochrein, F. Ampenberger, T. Gellert, H. Dietrich, G. Lipford, K. Takeda, S. Akira, H. Wagner, S. Bauer, The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily, Eur. J. Immunol. 33 (2003) 2987–2997.
[80] M. Matsumoto, K. Funami, M. Tanabe, H. Oshiumi, M. Shingai, Y. Seto, A. Yamamoto, T. Seya, Subcellular localization of Toll-like receptor 3 in human dendritic cells, J. Immunol. 171 (2003) 3154–3162.
[81] L. Alexopoulou, A.C. Holt, R. Medzhitov, R.A. Flavell, Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3, Nature 413 (2001) 732–738.
[82] K. Kariko, P. Bhuyan, J. Capodici, D. Weissman, Small interfering RNAs mediate sequence-independent gene suppression and induce immune activation by signaling through toll-like receptor 3, J. Immunol. 172 (2004) 6545–6549.
[83] F. Heil, H. Hemmi, H. Hochrein, F. Ampenberger, C. Kirschning, S. Akira, G. Lipford, H. Wagner, S. Bauer, Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8, Science 303 (2004) 1526–1529.
[84] H. Hemmi, O. Takeuchi, T. Kawai, T. Kaisho, S. Sato, H. Sanjo, M. Matsumoto, K. Hoshino, H. Wagner, K. Takeda, S. Akira, A toll-like receptor recognizes bacterial DNA, Nature 408 (2000) 740–745.
[85] A. Krug, A.R. French, W. Barchet, J.A. Fischer, A. Dzionek, J.T. Pingel, M.M. Orihuela, S. Akira, W.M. Yokoyama, M. Colonna, TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function, Immunity 21 (2004) 107–119.
[86] K. Honda, T. Taniguchi, IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors, Nat. Rev. Immunol. 6 (2006) 644–658.
[87] V.F. Van Tendeloo, P. Ponsaerts, Z.N. Berneman, mRNA-based gene transfer as a tool for gene and cell therapy, Curr. Opin. Mol. Ther. 9 (2007) 423–431.
[88] J. Banchereau, A.K. Palucka, Dendritic cells as therapeutic vaccines against cancer, Nat. Rev. Immunol. 5 (2005) 296–306.
[89] F. Grunebach, M.R. Muller, P. Brossart, RNA transfection of dendritic cells, Methods Mol. Med. 109 (2005) 47–54.
[90] E. Gilboa, J. Vieweg, Cancer immunotherapy with mRNA-transfected dendritic cells, Immunol. Rev. 199 (2004) 251–263.
[91] A. Bacci, C. Montagnoli, K. Perruccio, S. Bozza, R. Gaziano, L. Pitzurra, A. Velardi, C.F. d'Ostiani, J.E. Cutler, L. Romani, Dendritic cells pulsed with fungal RNA induce protective immunity to Candida albicans in hematopoietic transplantation, J. Immunol. 168 (2002) 2904–2913.
[92] H. Yu, L.A. Babiuk, S. van Drunen Littel-van den Hurk, Immunity and protection by adoptive transfer of dendritic cells transfected with hepatitis C NS3/4A mRNA, Vaccine 25 (2007) 1701–1711.
[93] E.R. Van Gulck, P. Ponsaerts, L. Heyndrickx, K. Vereecken, F. Moerman, A. De Roo, R. Colebunders, G. Van den Bosch, D.R. Van Bockstaele, V.F. Van Tendeloo, S. Allard, B. Verrier, C. Maranon, G. Hoeffel, A. Hosmalin, Z.N. Berneman, G. Vanham, Efficient stimulation of HIV-1-specific T cells using dendritic cells electroporated with mRNA encoding autologous HIV-1 Gag and Env proteins, Blood 107 (2006) 1818–1827.
[94] X. Zhou, P. Berglund, G. Rhodes, S.E. Parker, M. Jondal, P. Liljestrom, Self-replicating Semliki Forest virus RNA as recombinant vaccine, Vaccine 12 (1994) 1510–1514.
[95] H. Herweijer, J.S. Latendresse, P. Williams, G. Zhang, I. Danko, S. Schlesinger, J.A. Wolff, A plasmid-based self-amplifying Sindbis virus vector, Hum. Gene Ther. 6 (1995) 1161–1167.
[96] S. Schlesinger, Alphavirus vectors: development and potential therapeutic applications, Expert Opin. Biol. Ther. 1 (2001) 177–191.
[97] W.W. Leitner, L.N. Hwang, M.J. deVeer, A. Zhou, R.H. Silverman, B.R. Williams, T.W. Dubensky, H. Ying, N.P. Restifo, Alphavirus-based DNA vaccine breaks immunological tolerance by activating innate antiviral pathways, Nat. Med. 9 (2003) 33–39.
[98] J. Probst, B. Weide, B. Scheel, B.J. Pichler, I. Hoerr, H.G. Rammensee, S. Pascolo, Spontaneous cellular uptake of exogenous messenger RNA in vivo is nucleic acid-specific, saturable and ion dependent, Gene Ther. 14 (2007) 1175–1180.
[99] M. Szczepek, V. Brondani, J. Buchel, L. Serrano, D.J. Segal, T. Cathomen, Structure-based redesign of the dimerization interface reduces the toxicity of zinc-finger nucleases, Nat. Biotechnol. 25 (2007) 786–793.