Journal of Photochemistry and Photobiology B: Biology 129 (2013) 93–99

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Transfection of pseudouridine-modified mRNA encoding CPD-photolyase leads to repair of DNA damage in human keratinocytes: A new approach with future therapeutic potential

Gábor Boros [a], Edit Miko [a], Hiromi Muramatsu [b], Drew Weissman [c], Eszter Emri [a], Dávid Rózsa [a], Georgina Nagy [a], Attila Juhász [a], István Juhász [a,d], Gijsbertus van der Horst [e], Irén Horkay [a], Éva Remenyik [a,*], Katalin Karikó [b], Gabriella Emri [a]

[a] Department of Dermatology, Medical and Health Science Center, University of Debrecen, Nagyerdei korut 98, 4032 Debrecen, Hungary
[b] Department of Neurosurgery, University of Pennsylvania, 3450 Hamilton Walk, 371 Stemmler Hall, Philadelphia, PA 19104, USA
[c] Department of Medicine, University of Pennsylvania, 3610 Hamilton Walk, 522B Johnson Pavilion, Philadelphia, PA 19104, USA
[d] Department of Surgery and Operative Techniques, Faculty of Dentistry, Medical and Health Science Center, University of Debrecen, Nagyerdei korut 98, 4032 Debrecen, Hungary
[e] Department of Genetics, Section Chronobiology & Health, Erasmus University Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands

ARTICLE INFO

Article history:
Received 26 June 2013
Received in revised form 26 September 2013
Accepted 29 September 2013
Available online 11 October 2013

Keywords:
Pseudouridine-modified mRNA
mRNA therapy
Photolyase
Human keratinocyte
UVB
Cyclobutane pyrimidine dimers

ABSTRACT

UVB irradiation induces harmful photochemical reactions, including formation of Cyclobutane Pyrimidine Dimers (CPDs) in DNA. Accumulation of unrepaired CPD lesions causes inflammation, premature ageing and skin cancer. Photolyases are DNA repair enzymes that can rapidly restore DNA integrity in a light-dependent process called photoreactivation, but these enzymes are absent in humans. Here, we present a novel mRNA-based gene therapy method that directs synthesis of a marsupial, *Potorous tridactylus*, CPD-photolyase in cultured human keratinocytes. Pseudouridine was incorporated during *in vitro* transcription to make the mRNA non-immunogenic and highly translatable. Keratinocytes transfected with lipofectamine-complexed mRNA expressed photolyase in the nuclei for at least 2 days. Exposing photolyase mRNA-transfected cells to UVB irradiation resulted in significantly less CPD in those cells that were also treated with photoreactivating light, which is required for photolyase activity. The functional photolyase also diminished other UVB-mediated effects, including induction of IL-6 and inhibition of cell proliferation. These results demonstrate that pseudouridine-containing photolyase mRNA is a powerful tool to repair UVB-induced DNA lesions. The pseudouridine-modified mRNA approach has a strong potential to discern cellular effects of CPD in UV-related cell biological studies. The mRNA-based transient expression of proteins offers a number of opportunities for future application in medicine.

1. Introduction

Ultraviolet (UV) radiation, especially UVB (290–320 nm), is a common environmental carcinogen that induces harmful photochemical reactions in the skin [1]. UVB photons absorbed by the DNA cause a variety of photoproducts, including Cyclobutane Pyrimidine Dimers (CPDs), which is the most frequent and deleterious photolesion. It forms in double-stranded DNA when two adjacent pyrimidine bases in one strand fuse together [2]. Accumulated CPDs induce apoptosis, inflammation, immune suppression, and mutations that may lead to melanoma and other types of skin cancer [3–6]. Timely repair of damaged DNA is critical to prevent these adverse effects. In humans and rodents, photoproducts are removed by nucleotide excision repair (NER), a versatile repair system for removal of bulky DNA lesions. Genetic defects in NER result in photosensitive disorders, such as xeroderma pigmentosum and Cockayne's syndrome, in which patients have UV sensitivity and display skin cancer proneness and/or accelerated aging [7,8]. NER excises CPDs at a very low rate, requiring hours to days to restore DNA integrity [9–12]. Interestingly, in most organisms from bacteria to marsupials, CPDs are rapidly removed by photolyases that specifically recognize and split these dimers into monomers using energy from visible light [13–15]. Due to an evolutionary loss, photolyases are absent in placental mammals, including humans, which therefore solely rely on NER for removal of photolesions [16–19].

* Corresponding author. Tel.: +36 30 974 1545.
E-mail addresses: gaba@med.unideb.hu (G. Boros), mikoe4@gmail.com (E. Miko), hiromim@mail.med.upenn.edu (H. Muramatsu), dreww@mail.med.upenn.edu (D. Weissman), emeszti@gmail.com (E. Emri), davbiol@gmail.com (D. Rózsa), georginahn@gmail.com (G. Nagy), labor@berettyokorhaz.hu (A. Juhász), ji@med.unideb.hu (I. Juhász), g.vanderhorst@erasmusmc.nl (G. van der Horst), ihorkay@med.unideb.hu (I. Horkay), remenyik@med.unideb.hu (É. Remenyik), kariko@mail.med.upenn.edu (K. Karikó), gemri@med.unideb.hu (G. Emri).

1011-1344/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jphotobiol.2013.09.010

Studies have demonstrated that CPD photolyases from phylogenetically diverse organisms, such as cyanobacteria (*Anacystis nidulans*) [20], yeast [21] or marsupials [22], can function in mammalian cells. Liposome-encapsulated CPD photolyase enzyme from *A. nidulans* has been shown to protect cultured mammalian cells and human skin from the effects of UVB [20,23–26]. Superior resistance to UVB-induced sunburn, immune suppression and carcinogenesis were demonstrated for transgenic mice ubiquitously expressing CPD photolyase of the rat kangaroo (*Potorous tridactylus*) [22,27–29]. Mammalian cell lines stably expressing marsupial CPD photolyases also demonstrated reduced apoptosis and mutation frequencies when exposed to UVB radiation [30–33]. To study sunlight-induced DNA damage and their repair in primary cells, transient adenovirus-mediated CPD photolyase expression was used [34,35].

Gene therapy for the replacement of defective genes or for the expression of therapeutic proteins has made great progress in the last decade. After exploring numerous systems for expressing the encoded proteins [36], *in vitro* transcribed mRNA seems to be the most suitable tool for transient protein expression [37]. It has many features that make mRNA-mediated gene transfer especially valuable for functional characterization of encoded protein. The transfected mRNA is translated with high efficiency in any cell, including primary and non-dividing mammalian cells [37]. Importantly, when mRNA is delivered to the cell, only the encoded protein of interest is generated, unlike other vectors, such as plasmids that contain sequences for additional proteins, or viral vectors that not only code for but also carry viral proteins into the cell. In the last several years, mRNA-mediated transfection technology has improved tremendously [38]. It is now well documented that incorporation of pseudouridine (Ψ), a naturally-occurring modified nucleoside, into mRNA makes it less immunogenic by avoiding the activation of RNA sensors [39–41]. *In vitro*-transcribed mRNA containing pseudouridine (Ψ-mRNA) is also translated more efficiently than mRNA containing unmodified nucleosides [42]. We have demonstrated that the presence of Ψ nucleosides improved the stability of RNA [43] and diminished both activation of RNA-dependent protein kinase (PKR) and inhibition of translation [44]. A newly established preparative HPLC purification procedure was critical to obtain Ψ-mRNA that is free of aberrant transcription products, resulting in superior translational potential and no immunogenicity [45,46].

Here, we present a novel mRNA-based gene therapy method that directs functional photolyase synthesis in human keratinocytes. Our study demonstrates that delivery of HPLC-purified, Ψ-mRNA encoding CPD-photolyase into keratinocytes leads to the rapid repair of UVB-induced CPDs and suggest that such mRNA has therapeutic potential to repair damage caused by exposure to the sun and other sources of UV radiation.

2. Material and methods

2.1. RNA synthesis

A codon-optimized photolyase gene from *P. tridactylus* (rat kangaroo) containing GC-rich codons for superior translation was synthesized by Entelechon (Bad Abbach, Germany). The optimization increased the GC-content of the photolyase coding sequence (Accession: D26020) from 51.8% to 65.0%. Messenger RNAs encoding CPD-photolyase (CPD-PL Ψ-mRNA) and enhanced Green Fluorescent Protein (eGFP Ψ-mRNA) were transcribed as previously described [42] from linearized plasmids (pTEV-CPD-PL-A101 and pTEVeGFP-A101), using the Megascript T7 RNA polymerase kit (Ambion, Austin, TX) in which UTP was replaced with pseudouridine triphosphate (TriLink, San Diego, CA). Subsequently, the mRNA was HPLC purified as described [45], and provided with a 5′ cap using capping enzyme and 2′-O-methyltransferase according to the manufacturer (CellScript, Madison, WI). The RNA was transcribed to contain an encoded 101-nt long 3′ poly(A) tail, which was extended with ~300 nucleotides using poly(A) polymerase (USB, Cleveland OH). RNA samples were analyzed by agarose gel electrophoresis for quality assurance. The mRNAs were shown to be free of dsRNA contaminants using dsRNA-specific J2 mAb (English & Scientific Consulting, Budapest, Hungary) in a dot-blot assay [45]. The mRNAs were stored in siliconized tubes at −20 °C.

2.2. Cell cultures

The human keratinocyte cell line HaCaT was obtained from the ATCC and grown in high glucose DMEM (PAA, Traun, Austria) supplemented with 2 mM L-glutamine (PAA), 10% heat-inactivated fetal bovine serum (Lonza, Verviers, Belgium) and 0.5% antibiotic/antimycotic solution (Sigma–Aldrich, St. Louis, MO, USA) at 37 °C in a 5% CO_2 atmosphere. Normal Human Epidermal Keratinocytes (NHEK) were isolated from healthy adult skin derived from plastic surgery and cultured in EpiLife serum-free, complete keratinocyte growth medium (Life Technologies, Carlsblad, CA, USA). Ethics approval was received from the National Scientific and Research Ethics Commission. HaCaT cells and second passage NHEK were used at 70–80% confluency in each experiment.

2.3. Transient transfection and treatments

HaCaT cells and NHEK were seeded into 96-well plates at a density of 2×10^4 cells per well 1 day prior to transfection. Aliquots of RNA samples (0.25 μg) were complexed with 0.8 μl Lipofectamine LTX-PLUS (Life Technologies) in a final volume of 100 μl of EpiLife and the complexed RNA was added to each well. After 1 h the lipofectamine-RNA complex was replaced with 100 μl culture medium. At 20 h post transfection, cells covered with 50 μl Dulbecco's Phosphate Buffer Saline (DPBS) (Life Technologies) were subjected to 20 mJ/cm^2 UVB using two TL-20 W/12 bulbs (Philips). Immediately after UVB treatment, cells were either exposed to visible light using a F18W Daylight fluorescent bulb (Sylvania,) at a distance of 16 cm and using a 4 mm thick glass filter as a shield or kept in the dark for 1 h. Cells were further cultured in complete medium for the indicated times. Cell viability was determined by the EZ4U assay (Biomedica Gruppe, Vienna, Austria), according to the manufacturer.

2.4. Western blot analysis

Cells were lysed in RIPA buffer (R0278, Sigma–Aldrich) containing protease inhibitor cocktail (Sigma–Aldrich). Proteins were separated on 10% polyacrylamide gels and transferred to nitrocellulose membranes. The membrane was blocked in 5% non-fat dry milk for 1 h and incubated overnight at 4 °C with primary CPD-photolyase antibody [27] diluted (1:500) in PBGTNa made of PBS containing 0.5% bovine serum albumin (BSA, Amresco, Solon, OH, USA), 0.05% gelatin (Sigma–Aldrich), 0.05% Tween-20 (Amresco) and 300 mM NaCl (VWR, San Diego, CA, USA). HRP-conjugated anti-rabbit IgG was used as a secondary antibody (1:3000; 1 h, at room temperature, BioRad, Berkeley, CA, USA). Proteins were visualized with the Pierce ECL Plus Western blotting detection system (Thermo Fisher Scientific, Rockford, IL, USA).

2.5. Fluorescence microscopy

Expression of eGFP in HaCaT cells was documented using an epifluorescent Zeiss Axiovert 100 microscope mounted with a Zeiss