DNA REPAIR 5 (2006) 925–934

available at www.sciencedirect.com

SCIENCE DIRECT®

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

Adenovirus mediated transduction of the human DNA polymerase eta cDNA

Keronninn Moreno Lima-Bessa[a], *Vanessa Chigançasb*[b], *Anne Stary*[b], *Patricia Kannouche*[b], *Alain Sarasin*[b], *Melissa Gava Armelini*[a], *Jacqueline de Fátima Jacysyn*[c], *Gustavo P. Amarante-Mendes*[c], *Marila Cordeiro-Stone*[d], *James E. Cleaver*[e], *Carlos Frederico Martins Menck*[a,*]

[a] *Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 1374 São Paulo, SP 05508-900, Brazil*

[b] *Laboratory of Genetic Instability and Cancer, Institut Gustave-Roussy, Villejuif, France*

[c] *Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil*

[d] *Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7525, USA*

[e] *Auerback Melanoma Laboratory, Room N431, UCSF Cancer Center, Box 0808, Room N431, UCSF Cancer Center, University of California, San Francisco, CA 94143-0808, USA*

ARTICLE INFO

Article history:
Received 9 February 2006
Received in revised form 8 May 2006
Accepted 16 May 2006
Published on line 23 June 2006

Keywords:
DNA repair
Recombinant adenovirus
Xeroderma pigmentosum
Ultraviolet light
DNA polymerase eta

ABSTRACT

Xeroderma pigmentosum (XP) is an autosomal recessive photosensitive disorder with an extremely high incidence of skin cancers. Seven complementation groups, corresponding to seven proteins involved in nucleotide excision repair (NER), are associated with this syndrome. However, in XP variant patients, the disorder is caused by defects in DNA polymerase η; this error prone polymerase, encoded by *POLH*, is involved in translesion DNA synthesis (TLS) on DNA templates damaged by ultraviolet light (UV). We constructed a recombinant adenovirus carrying the human *POLH* cDNA linked to the *EGFP* reporter gene (Ad*XPV-EGFP*) and infected skin fibroblasts from both XPV and XPA patients. Twenty-four hours after infection, the DNA polymerase η-EGFP fusion protein was detected by Western blot analysis, demonstrating successful transduction by the adenoviral vector. Protein expression was accompanied by reduction in the high sensitivity of XPV cells to UV, as determined by cell survival and apoptosis-induction assays. Moreover, the pronounced UV-induced inhibition of DNA synthesis in XPV cells and their arrest in S phase were attenuated in Ad*XPV-EGFP* infected cells, confirming that the transduced polymerase was functional. However, over-expression of polymerase η mediated by Ad*XPV-EGFP* infection did not result in enhancement of cell survival, prevention of apoptosis, or higher rate of nascent DNA strand growth in irradiated XPA cells. These results suggest that TLS by DNA polymerase η is not a limiting factor for recovery from cellular responses induced by UV in excision-repair deficient fibroblasts.

© 2006 Elsevier B.V. All rights reserved.

* *Corresponding author*. Tel.: +55 11 3091 7499; fax: +55 11 3091 7354.
E-mail address: cfmmenck@usp.br (C.F.M. Menck).

1568-7864/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2006.05.037

1. Introduction

Cellular DNA is continuously exposed to a large variety of DNA-damaging agents, and all living organisms are equipped with DNA repair systems responsible for removal of lesions and maintenance of genetic integrity [1]. The repair processes, however, are in some cases slow and incomplete, forcing cells to replicate the genome containing persistent damage, and special cell machineries have also evolved to undertake this function.

Ultraviolet (UV) light, present in solar radiation, is known to induce two major types of DNA lesions: cyclobutane–pyrimidine-dimers (CPDs) and pyrimidine–(6-4)-pyrimidone photoproducts (6-4PPs). These lesions cause distortions in the DNA double helix and inhibit essential cellular processes, such as DNA replication and RNA transcription. UV-induced DNA photoproducts have been extensively studied and implicated in skin carcinogenesis.

Xeroderma pigmentosum (XP) is an autosomal recessive syndrome characterized by hypersensitivity to UV-induced DNA damage, resulting in high frequency of skin cancer, even in moderate exposure to solar radiation [2]. Most XP patients are defective in nucleotide excision repair (NER), the pathway that recognizes and removes bulk lesions from the genome, such as UV-induced DNA photoproducts [3]. XP variant (XPV) patients, accounting for approximately 25% of XP cases, do not present any abnormality in NER pathway, but are affected in their capacity to replicate DNA after UV-irradiation [4]. Although the existence of the variant group of XP patients has been known for more than 3 decades [5,6], only recently the gene responsible for the XPV phenotype has been identified: *POLH,* the gene that encodes DNA polymerase η (polη) [7,8].

Polη belongs to the Y-family of mammalian DNA polymerases involved in translesion DNA synthesis (TLS), a damage-tolerance mechanism that supports the direct bypass of template DNA lesions, which cannot be accomplished by the high-fidelity DNA polymerases [9,10]. Polymerases belonging to Y-family (polη, polι, polκ and Rev1) are characterized by lower processivity and high frequency of nucleotide mis-incorporation *in vitro* [11,12]. Interestingly, XPV cells are known for their hypermutable phenotype [13], suggesting that few errors are made by polη during the TLS process and favoring the hypothesis that polη is a relatively accurate DNA polymerase when replicating UV-damaged templates [14].

Polη is a protein of 713 aminoacids, but its polymerase activity is contained within 511 aminoacids of the N-terminal region [7]. However, XPV patients expressing polη with intact polymerase activity and truncated C-terminus were found, indicating that the latter region plays an important functional role. Analysis of this region of polη has shown the presence of at least two important domains: the first one corresponds to approximately 70 aminoacids and contains a nuclear localization signal and the other, with approximately 50 aminoacids, is responsible for directing polη to replication foci after UV-irradiation [15]. A putative third motif in the C-terminus region of polη seems to include a zinc-finger. Although the presence of this motif still needs to be confirmed, it is conserved from yeasts to humans. *In vitro*, this motif is not required for the polymerase activity of polη, but it could have an important role *in vivo*, increasing its binding to DNA when replication forks are blocked by UV photolesions [15].

Although *in vitro* experiments have shown that polη can replicate DNA templates containing other types of lesions, CPDs appear to be its main target lesions [7,8,14]. In contrast to most TLS polymerases, polη has been found to bypass CPDs efficiently and accurately. Polη accuracy *in vitro* seems to result from both its capacity to select the correct nucleotide during TLS and its failure to elongate DNA chains with mispaired 3′-termini [14]. Interestingly, structural analysis of the Rad30 protein, polη homologue in *Saccharomyces cerevisiae*, revealed the presence of a much more open active site in this protein, compared to replicative DNA polymerases. This site might accommodate two nucleotides at the same time and this characteristic could explain why this polymerase can replicate through CPDs [16]. Additional experiments showed that polη usually inserts two adenines opposite thymine dimers, the CPD most frequently formed in UV-irradiated DNA; these findings provide an explanation for why mutagenesis at TT sites is low and why mutations induced by UV-irradiation are preferentially located at dipyrimidine sites containing cytosines [17,18].

In recent years, adenoviral vectors have been used for research and therapeutic purposes. Adenoviral vectors have some potential advantages when compared to other vectors, such as they can be easily manipulated *in vitro* and are normally produced at very high titers. Furthermore, these viruses are able to infect a wide variety of cell types, including differentiated and non-dividing cells. These characteristics make adenoviral vectors powerful tools for efficient transduction of foreign DNA into human cells [19,20]. We have constructed a recombinant adenovirus carrying human *POLH* cDNA linked to the EGFP reporter gene (Ad*XPV-EGFP*). XPV cells infected with this adenoviral vector displayed higher UV-resistance than non-infected ones, as determined by increased cell survival, reduced apoptosis, decreased S-phase arrest and increased rate of DNA elongation after UV-irradiation. Taken together, these results indicated that polη-EGFP is functional and efficiently corrected the XPV cells' defect. On the other hand, XPA cells over-expressing polη (endogenous polη plus polη-EGFP from Ad*XPV-EGFP*) did not show any improvement in UV-sensitivity (apoptosis and cell survival) or DNA synthesis after UV-irradiation. These results confirmed that polη contributes to preventing cell death triggered by UV-DNA damage in human cells, but it cannot overcome the deficiency in the removal of those lesions by nucleotide excision repair.

2. Materials and methods

2.1. Cell lines and culture conditions

SV40-transformed skin fibroblasts were isolated from skin biopsies of XPV (XP30RO-SV) and XPA (XP12RO-SV) patients. MRC5-V1 fibroblasts display normal TLS on UV-damaged templates, as well as NER, and were used as a positive con-

Download English Version:

https://daneshyari.com/en/article/1981395

Download Persian Version:

https://daneshyari.com/article/1981395

Daneshyari.com