RESEARCH NOTE

MYCOBACTERIUM TUBERCULOSIS uvrC ESSENTIALITY IN RESPONSE TO UV-INDUCED CELL DAMAGE

Therdsak Prammananan[1], Saranya Phunpruch[2], Sarinya Jaitrong[1] and Prasit Palittapongarnpim[1,3]

[1]TB Research Laboratory, Medical Molecular Biology Research Unit, National Center for Genetic Engineering and Biotechnology, Thailand Science Park, Pathum Thani; [2]Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Lat Krabang, Bangkok; [3]Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand

Abstract. Control of tuberculosis depends both on an effective, accurate, and rapid diagnosis and an effective treatment and management. Antituberculous drugs have been used for more than 50 years and are likely ineffective against multidrug-resistant strains, leading to an urgent need for new drugs. Comparative genome analysis has indicated that *Mycobacterium tuberculosis uvrC*, a component of nucleotide excision repair (NER) system, is an essential gene without any human homolog. This raises the possibility to use this gene as a new drug target. This study investigated the essential role of *uvrC* on growth of *M. tuberculosis* in the presence of DNA damaging agents, UV light and hydrogen peroxide (generator of reactive oxygen species). Results revealed that the *M. tuberculosis uvrC* mutant was more sensitive to UV than the control strain ($p<0.01$), but was not more sensitive to hydrogen peroxide. These results showed that *uvrC* is essential for *M. tuberculosis* DNA repair system, particularly in response to DNA damage caused by UV irradiation.

Keywords: *Mycobacterium, uvrC*, excision repair, UV, oxidative stress

INTRODUCTION

The increasing incidence of multidrug-resistant tuberculosis (MDR-TB) has led to an urgent need for new anti-TB drugs. Other than screening of natural compounds, which contain antituberculous activity, studies identifying new potential drug targets are alternative strategies to provide valuable information needed for development of new drugs. Preliminary study using minimal genome comparison has identified a group of essential genes without human homolog in *Mycobacterium tuberculosis* (Thammarongtham and Palittapongarnpim, 2002). *M. tuberculosis* Rv1420, annotated as

Correspondence: Therdsak Prammananan, TB Research Laboratory, Medical Molecular Biology Research Unit, National Center for Genetic Engineering and Biotechnology, Thailand Science Park, Pathum Thani 12120, Thailand.
Tel: +66 (0) 2419 8256; Fax: +66 (0) 2418 2094
E-mail: therdsak@biotec.or.th

Table 1
Primers used for constructing antisense *recA* and *uvrC* sequences.

Gene	Primer	Sequence (5′→3′)
recA (Rv2737c)	RAUP-f	GCTAGCGGTGTTGAGCAGAT
	RAUP-r	CGAGCTTCTTGGCATAGTCC
uvrC (Rv1420)	UCUP-f	GCGTGAATATCGGAAATGCT
	UCPU-r	GTATTCCAGCTGCAGTGCCT

uvrC homolog, is included in this group. This gene is highly conserved among the *Mycobacterium* species, but its function still has not been investigated hitherto in mycobacteria.

The nucleotide excision repair (NER) system is relatively nonspecific and can repair many different types of DNA damage (Eisen and Hanawalt, 1999). This type of repair recognizes distortions in the normal DNA helix that result from the damage, rather than the chemical nature of the damage itself. In general, NER can be viewed as consisting of four basic steps: i) damage recognition and lesion verification by UvrA and UvrB, ii) incision by UvrC, iii) excision by UvrD, and iv) repair synthesis and ligation by DNA polymerase I and ligase, respectively (van Houten *et al*, 2005).

Amino acid alignment with *E. coli* UvrC indicates that the putative *M. tuberculosis* UvrC should play a similar role in NER as that in *E. coli*. Although genes involved in NER system, viz. *uvrA*, *uvrB*, *uvrC*, and *uvrD* have been shown to be upregulated in *M. tuberculosis* within human macrophage (Graham and Clark-Curtiss, 1999), only *uvrB* and *uvrD* have been intensively studied in *M. tuberculosis* (Darwin and Nathan, 2005; Güthlein *et al*, 2009). The UvrB mutant strain is hypersensitive to UV and nitric oxide but not to reactive oxygen species (ROS) *in vitro* and is attenuated in mice. These results suggest the essentiality of Uvr system in *M. tuberculosis* for intracellular survival, raising the possibility of targeting this system for experimental chemotherapy. The present study, therefore, sought to validate another component of the system, UvrC, for its essential role in *M. tuberculosis*.

MATERIALS AND METHODS

Construction of UvrC *M. tuberculosis* mutant

Gene silencing of *uvrC* was performed using antisense strategy. A 521-bp DNA fragment consisting of 272-bp upstream and 249-bp coding sequences of *uvrC* of *M. tuberculosis* H37Rv was amplified by PCR. Primers were designed based on *M. tuberculosis* H37Rv complete genome deposited in GenBank database (Table 1). The antisense plasmid, pMV261-uvrC(AS) was constructed by subcloning the 521-bp PCR amplicon into the replicative plasmid pMV261 (generating 3-5 copies of cloned DNA per replication) (Stover *et al*, 1991) in an antisense direction. An antisense plasmid containing 582-bp DNA fragment of *M. tuberculosis recA* (257-bp upstream and 325-bp coding sequences), pMV261-recA(AS), was also constructed as the same manner and used as a positive gene-silencing control. All plasmids,

including the parental pMV261 (as a negative control), were transfected into *M. tuberculosis* H37Ra ATCC 25177 by electroporation (Parish and Stoker, 1998). The transformants obtained carrying each plasmid were investigated for growth in the presence of UV-light and ROS generated by H_2O_2.

Susceptibility test to UV irradiation

Exponential growth cultures of *M. tuberculosis* H37Ra containing either pMV261-uvrC(AS), pMV261-recA(AS) or pMV261 were adjusted to the turbidity comparable to a McFarland No.1 standard (approximately $3x10^7$ cells/ml). UV exposure (intensity of 2.48 mW/cm^2) was performed using a microtiter plate with 200 l suspension per well. The microtiter plate was placed under the UV lamp (PATCO UV T5 tube) (6 W placed 8 cm above the plates) with different exposure times (0, 1, 2, 5, 10 and 20 minutes). The colony forming units (CFUs) of each time point were determined by plating 200 l of 10-fold serial dilutions of each suspension onto M7H10-OADC plates containing 25 g/ml kanamycin (triplicate per dilution). All plates were sealed with plastic wrap and incubated at 37°C for 3-4 weeks. Experiments were conducted in three independent occasions.

Susceptibility test to H_2O_2

Exponential growth cultures of *M. tuberculosis* H37Ra containing either pMV261-uvrC(AS), pMV261-recA(AS) or pMV261 were adjusted to the turbidity comparable to a McFarland No.1 standard (approximately $3x10^7$ cells/ml). One ml bacterial suspension was exposed to 5, 10 and 20 mM H_2O_2 (Merck, Darmstadt, Germany) for 3 hours at 37°C. CFU of each treatment was determined as described above. Experiments were conducted in three independent occasions.

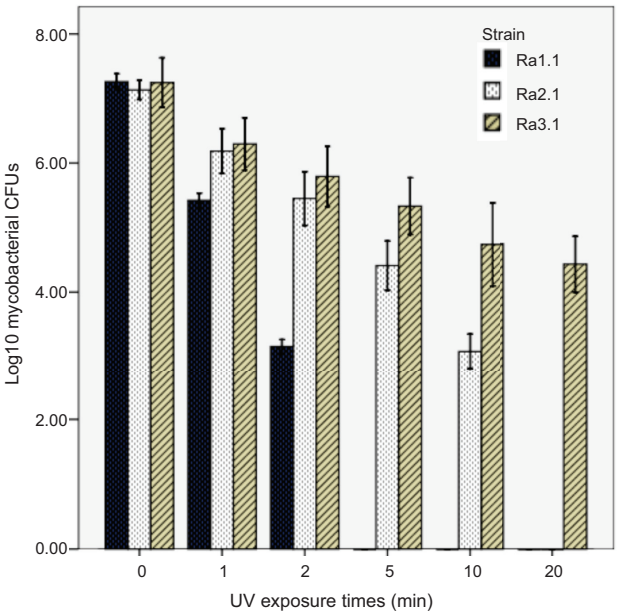

Fig 1–Susceptibility to UV of overexpressed-antisense *M. tuberculosis* H37Ra strains. Ra1.1, *M. tuberculosis* H37Ra::pMV261-recA(AS); Ra2.1, *M. tuberculosis* H37Ra::pMV261-uvrC(AS) and Ra3.1, *M. tuberculosis* H37Ra::pMV261. Difference in CFUs is significant between *recA* mutant (Ra1.1), and between *uvrC* mutant (Ra2.1) and control strain (Ra3.1), respectively (p<0.01).

Statistical analysis

Results are presented as mean± S.E.M. CFU of three independent experiments. SPSS for Windows version 13.0 (SPSS, Chicago, IL) was used for statistical analysis. Differences between groups were tested by 2 ways-ANOVA. A p-value < 0.05 was considered statistically significant.

RESULTS

Exponential growth cultures of *M. tuberculosis* H37Ra carrying pMV261-recA(AS) [Ra1.1], pMV261-uvrC(AS) [Ra2.1], and pMV261 [Ra3.1] were irradi-

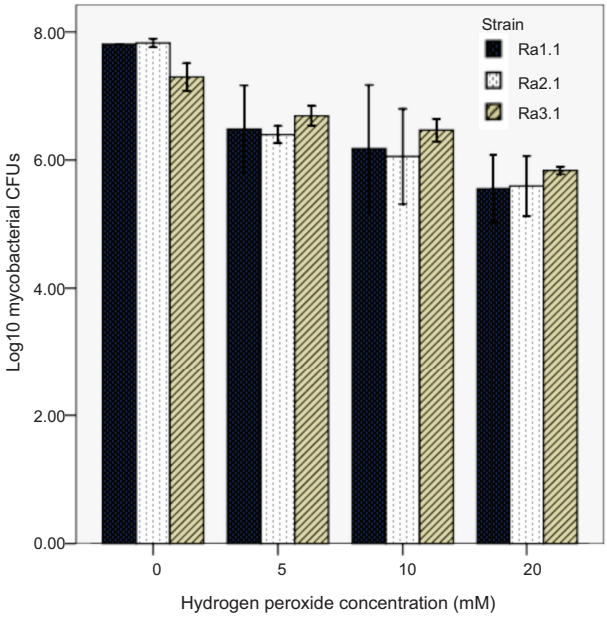

Fig 2–Susceptibility to H_2O_2 of the overexpressed-antisense *M. tuberculosis* H37Ra strains. Ra1.1, *M. tuberculosis* H37Ra::pMV261-recA(AS); Ra2.1, *M. tuberculosis* H37Ra::pMV261-uvrC(AS) and Ra3.1, *M. tuberculosis* H37Ra::pMV261.

ated by short-wavelength UV for upto 20 minutes and CFU was determined. Strains carrying antisense *recA* and *uvrC* were more susceptible to UV than the control strain (Fig 1).

Transformed *M. tuberculosis* strains carrying antisense *recA* and *uvrC* were investigated for their susceptibility to H_2O_2. Exponential growth cultures of *M. tuberculosis* H37Ra carrying pMV261-recA(AS) [Ra1.1], pMV261-uvrC(AS) [Ra2.1], and pMV261 [Ra3.1] were exposed to 5-20 mM H_2O_2 for 3 hours and CFU was determined. No statistical significances are found among the three strains tested (Fig 2).

DISCUSSION

In silico analysis of mycobacterial genome including *M. tuberculosis* revealed the presence of genes encoding enzymes involved in DNA repair systems, such as DNA damage reversal, NER, base excision repair (BER), recombinational repair, nonhomologous end joining, and SOS repair (Cole *et al*, 1998; Mizrahi and Andersen, 1998). Interestingly, *M. tuberculosis* lacks the *mutLS*-based postreplicative mismatch repair (MMR) system, which is highly conserved throughout evolution and prevents mutation by correcting replication errors (Mizrahi and Andersen, 1998; Springer *et al*, 2004). In addition, MMR inhibits homologous recombination and therefore helps to control the fidelity of recombination (Rayssiguier *et al*, 1989; Worth *et al*, 1994; Matic *et al*, 1995).

In fact, mycobacteria exhibit comparable mutation rate similar to other MMR-proficient bacteria, suggesting that they possess alternative or compensating mechanisms for mismatch recognition and MMR (Springer *et al*, 2004). It is therefore possible that the fidelity of replication and recombination is controlled by one of the other DNA repair system. For example, NER is very efficient and seems to be common to most types of organisms (Eisen and Hanawalt, 1999). It is also relatively nonspecific and will repair many different types of damage including damage caused by UV irradiation, *eg*, cyclobutane dimmers, 6-4 lesions, and base-sugar cross-links. Mutation or deficiencies in specific NER genes can lead to enhanced mutagenesis, hypersensitivity to DNA damaging agents, and attenuated phenotype in bacteria (Maragos *et al*, 1993; Darwin and Nathan, 2005). Prokaryotic NER has been extensive studied in *E. coli* and it is mediated by UvrABC excinucle-

ase enzyme complex and helicase UvrD (Sancar, 1996).

Investigations of UvrB and UvrD of NER system in *M. smegmatis* revealed that the *uvrB* mutant, as well as *uvrD* mutant, was more sensitive to UV (short-wavelength) irradiation than the wild type strain (Güthlein *et al*, 2009). Similarly, both mutants are more sensitive to ROS generated by *tert*-butylhydroperoxide (TBH) and reactive nitrogen intermediates (RNI) generated by sodium nitrite than the wide type strain (Güthlein *et al*, 2009). However, the *uvrC* has not hitherto been studied in mycobacteria.

Our study demonstrated the successful use of antisense plasmid for interference of a gene function. The antisense sequences of *recA* (hypersensitive to UV and used as positive control) and *uvrC* were cloned and over-expressed in *M. tuberculosis* using the replicative plasmid that generates 3-5 copies of cloned DNA per replication. The *uvrC* mutant, as well as the *recA* mutant, was more sensitive to UV irradiation than the control strain. In contrast, all strains showed no difference in susceptibility to ROS generated by H_2O_2. The *uvrC* mutant showed UV hypersentivity similar to the *recA* mutant, indicating that it plays a crucial role on DNA repair response to damage by UV, in agreement with the previous study of *uvrB* and *uvrD* mutant that are UV hypersensitive as well (Güthlein *et al*, 2009). On the other hand, the *uvrC* mutant was not more sensitive to ROS as previously demonstrated for *uvrB* and *uvrD* mutants. It could explain either by that i) if mycobacterial NER is essential for repairing DNA damage by ROS as shown by the effects of *uvrB* and *uvrD* mutants, it is possible that there is another protein(s) that can compensate the function of UvrC, or that ii) with our silencing technique complete *uvrC* null mutant was not obtained and the remaining UvrC could still function sufficiently for bacterial survival under the test conditions.

Together with evidences from other studies, it suggests that the mycobacterial NER plays an essential role on repairing DNA damage with a wider range of damage than the *E. coli* system and that the UvrC, a key component of NER, was shown in this study to be essential, at least, for repairing DNA damage from UV irradiation.

ACKNOWLEDGEMENTS

The authors would like to thank all staff of the Drug-Resistant Tuberculosis Research Laboratory, Drug-Resistant Tuberculosis Research Fund for their technical assistance. The study was funded by CPMO (P-00-20034), National Science and Technology Development Agency (NSTDA), Thailand.

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