Chapter 16

Screening for Suppression of Inflammatory Responses Against UVB-Induced DNA Damage in Skin Cells Based on Natural Plant Extract Enhances DNA Repair-Related Polymerase Activity

Sawako Shiratake, Takefumi Onodera,
Yuka Sakamoto, Tatsuo Nakahara,
Hiroyasu Iwahashi and Yoshiyuki Mizushina

Additional information is available at the end of the chapter

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1. Introduction

DNA-dependent DNA polymerase (Pol) (E.C. 2.7.7.7) catalyzes the polymerization of deoxyribonucleotides along a DNA strand that is "read" as a template [1]. The newly polymerized molecule is complementary to the template strand. Pol adds free nucleotides only to the 3′ end of a newly formed strand, resulting in elongation of the new strand in the 5′ → 3′ direction. The human genome encodes at least 15 Pols that function in cellular DNA synthesis (Table 1) [2-5]. Eukaryotic cells contain three replicative Pols (α, δ, and ε), one mitochondrial Pol (γ), and at least 11 non-replicative Pols (β, ζ, η, θ, ι, κ, λ, μ, and ν), REV1, and terminal deoxynucleotidyl transferase (TdT) [2-5]. Pols have a highly conserved structure, with their overall catalytic subunits showing little variation among species. Conserved enzyme structures are usually preserved over time because they undertake important cellular functions that confer evolutionary advantages. Based on sequence homology, eukaryotic Pols can be divided into four main families: A, B, X, and Y [2-5]. Family A includes mitochondrial Pol γ as well as Pols θ and ν; family B includes the three replicative Pols α, δ, and ε, and Pol ζ; family X is comprised of Pols β, λ, and μ, as well as TdT; and family Y includes Pols η, ι, and κ, in addition to REV1 [5]. At least two Pols, such as B-family Pol ζ and Y-family REV1, have substantial translesion DNA synthesis (TLS) activity [5]. The most notable TLS Pol that bypasses ultraviolet (UV) radiation-induced DNA damage is Pol η, which bypasses TT-*cis*-syn cyclobutane pyrimidine

dimmers (TT-CPD) with high efficiency and fidelity. Purified human Pol η correctly inserts A deoxynucleotides opposite linked bases of a TT-CPD [6]. Pol β, the base excision repair (BER) Pol, enhances UV-induced genetic instability, and facilitates translesion replication of CPD in a UV lesion bypass [7]. Consequently, activation of DNA repair-related Pols β and η is likely important for maintaining UVB-induced DNA damage.

Name	Catalytic subunit		Family [a]	Function	KCl (120 mM) inhibition
	Gene	Size of protein (kDa)			
Pol α	*POLA1*	166	B	DNA replication priming	+
Pol β	*POLB*	38	X	BER and meiotic recombination	–
Pol γ	*POLG*	140	A	Mitochondrial DNA replication and repair	–
Pol δ	*POLD1*	124	B	DNA replication, NER, and MMR	+
Pol ε	*POLE*	262	B	DNA replication, NER, and MMR	+
Pol ζ	*REV3L*	353	B	TLS and mutagenesis	–
Pol η	*POLH*	78	Y	Bypass of UV radiation-induced DNA adducts, especially CPDs	–
Pol θ	*POLQ*	290	A	Defense against ionizing radiation-induced DNA damage	–
Pol ι	*POLI*	80	Y	Backup enzyme for UV radiation-induced DNA adducts and BER	–
Pol κ	*POLK*	99	Y	Bypass of bulky adducts, backup enzyme for NER	–
Pol λ	*POLL*	63	X	V(D)J recombination; possibly and joining	–
Pol μ	*POLM*	55	X	V(D)J recombination; possibly and joining	–
Pol ν	*POLN*	100	A	? (ICL repair or testis-specific function)	–
REV1	*REV1*	138	Y	TLS and mutagenesis, anchor for several Pols	–
TdT	*DNTT*	58	X	Immunoglobulin diversity at junctions of coding regions	–

BER, base excision repair; CPD, cyclobutane pyrimidine dimer; ICL, interstrand crosslink; MMR, mismatch repair; NER, nucleotide excision repair; Pol, DNA polymerase; TdT, terminal deoxynucleotidyl transferase; TLS, translesion DNA synthesis; UV, ultraviolet.

[a] In human cells, these enzymes fall into four distinct families, designated A, B, X, and Y, based on amino acid sequence.

This table was composed based on previous references [1, 5].

Table 1. Human Pol species.

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The skin is the largest organ of the human body and protects against external physical, chemical, and biological insults, including UV radiation and microorganisms. Although many environmental and genetic factors contribute to the development of various skin diseases, one of the most important factors is chronic exposure of the skin to solar UV radiation. Excessive exposure of the skin to UV radiation has many biological consequences, including sunburn, hyperpigmentation, solar keratosis, solar elastosis, skin cancer, immunosuppression, and acute inflammatory responses [8, 9]. UVB (290–320 nm) radiation induces keratinocyte apoptosis, which is evident within the epidermis as sunburn cells. The formation of sunburn cells in UVB-exposed skin reflects the severity of DNA damage. UV absorption produces two main types of DNA damage: CPD and pyrimidone photoproducts. However, the repair of DNA damage in UVB-exposed skin cells prevents accumulation of damaged cells [10]. UV-induced DNA damage is also an important molecular trigger for UV-induced inflammation, as well as various forms of skin cancer [11].

Sunscreens are commonly used for preventing or ameliorating harmful effects of UV radiation on the skin [12]. However, sunscreen alone may not provide sufficient protection against skin photodamage [13]. Non-sunscreen compounds have become more relevant to large parts of the population in preventative skin care [14]. Active compounds that support skin defensive mechanisms or inhibit pathological processes in photodamaged skin are highly desirable. Some plant extracts are reported to protect skin against various UV-induced damage [15], and there has been considerable interest in applying plant polyphenols to the prevention of UV-induced skin photodamage [16].

Therefore, we asked whether components from tropical plants, which absorb strong UV-containing sunlight, might enhance UV-damaged DNA repair-related Pols. In addition, we have been screening mammalian Pol inhibitors from natural products, such as food materials, nutrients, and higher plants, for over 20 years, and have identified more than 100 low-molecular-weight organic compounds as Pol inhibitors [17-19]. Through this process, we have developed a simple *in vitro* assay to screen for mammalian Pol inhibitors [20-22]. Therefore, we initially established an *in vitro* Pol enhancer assay using cell extracts from cultured normal human epidermal keratinocytes (NHEK) using this method. Next, we screened 50 tropical plant extracts for Pol enhancer activity in cultured NHEK, and purified the activity-enhancing compounds from extracts of the most bioactive plants. In this review, we explore the relationship between the DNA repair of UVB-stimulated DNA damage by cellular Pol activity in NHEK and the immunosuppression of UVB-irradiated skin cells treated with bioactive plant extract compounds.

2. Pol assay in UVB-irradiated NHEK

2.1. NHEK cultures

NHEK and serum-free keratinocyte growth medium (KGM, trade names: EpiLife-KG2 and HuMedia-KG2) containing insulin, hydrocortisone, gentamycin/amphotericin B, and growth additives such as bovine pituitary extract and human epidermal growth factor were purchased

from Kurabo Industries Ltd. (Osaka, Japan). NHEK were seeded at 3×10^5 cells/cm^2 into 75-cm^2 cell culture flasks, and cultured in KGM at 37°C under 5% CO_2. Third- or fourth-passage cells were used for all experiments. Test compounds, such as plant extracts and purified compounds, were dissolved in dimethyl sulfoxide (DMSO) and diluted with medium to appropriate concentrations, and the final volume adjusted to 0.05% (v/v) DMSO. NHEK were initially treated with test compound for 24 h (Figure 1). Cultures were then washed with Hank's buffer, irradiated with UVB (0–150 mJ/cm^2), and cultured for 2–24 h in KGM. After treatment, cultured cells were collected by a cell scraper, and sonicated for 10 s in lysis buffer containing 50 mM Tris–HCl (pH 7.5), 1 mM EDTA, 5 mM 2-mercaptoethanol, 15% glycerol, and a protease inhibitor cocktail of Complete Mini (Roche Diagnostics, Mannheim, Germany). Cell extract Pol activity was assayed and quantified *in vitro* as described previously [20-22] with some modification, as described below.

2.2. Measurement of *in vitro* Pol activity

For Pol reactions, poly (dA)/oligo(dT)$_{18}$ and [^{3}H]-labeled 2′-deoxythymidine-5′-triphosphate (dTTP; 43 Ci/mmol) were used as the DNA template-primer substrate and nucleotide (dNTP; 2′-deoxynucleoside-5′-triphosphate) substrate, respectively (Figure 1). A chemically synthesized DNA template, poly (dA), was purchased from Sigma-Aldrich Inc. (St Louis, MO, USA), and a customized oligo (dT)$_{18}$ DNA primer was purchased from Sigma-Aldrich Japan K.K. (Hokkaido, Japan). Radioactive nucleotides [^{3}H]-dTTP were obtained from Moravek Biochemicals Inc. (Brea, CA, USA). The standard reaction mixture for all Pol species (24 μL of final volume) contained 50 mM Tris–HCl, pH 7.5, 1 mM dithiothreitol, 1 mM $MgCl_2$, 5 μM poly (dA)/oligo(dT)$_{18}$ (A/T, 4:1), 10 μM [^{3}H]-dTTP (100 cpm/pmol), and 15% (v/v) glycerol. The standard reaction mixture for DNA repair-related Pol species was the same, except that it also contained 120 mM KCl. Cultured NHEK cell extract (16 μL) was mixed with 8 μL of standard Pol reaction mixture. After incubation at 37°C, for 60 min, the radioactive DNA product was collected on a DEAE–cellulose paper disc (DE81) as described by Lindell *et al.* [23], and the radioactivity measured in a scintillation counter (2300TR TriCarb; PerkinElmer, Downers Grove, IL, USA).

2.3. UVB enhancement of NHEK Pol activity

We first investigated whether cultured NHEK Pol activity was stimulated by UVB radiation. UVB at 12.5 and 25 mJ/cm^2 had no effect on Pol activity, whereas 50 mJ/cm^2 significantly enhanced activity (Figure 2A). Irradiation (150 mJ/cm^2) lead to the largest increase in Pol activity, with 1.59- and 1.78-fold enhanced activity for standard reaction mixtures without or with KCl, respectively. All 15 human Pols are active in the absence of salt (i.e., KCl and NaCl), whereas DNA replicative Pols, such as Pols α, δ, and ε, are inhibited by salt [1] (Table 1). Therefore, the standard reaction mixture for all Pols containing both DNA replication and repair Pols, or DNA repair-related Pols only, was with or without 120 mM KCl, respectively. Because the activity of DNA repair-related Pols (with KCl) is higher than all other Pol species (without KCl), the UV-damaged Pols, especially Pols β and η, might be enhanced in NHEK.

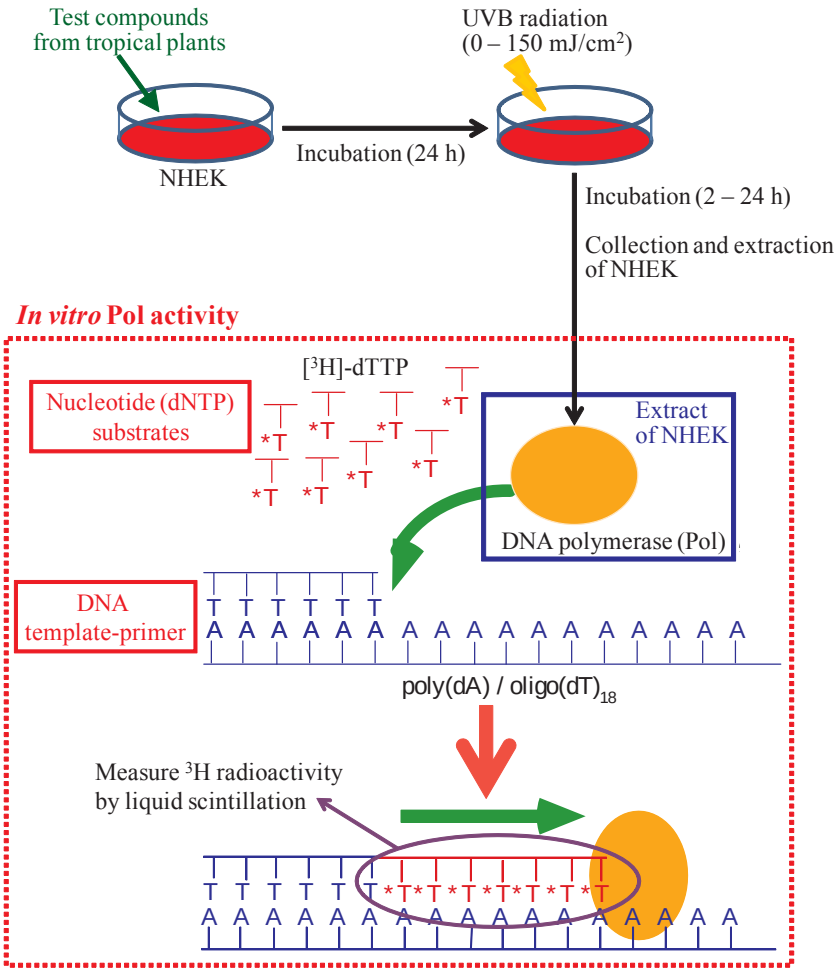

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Figure 1. *In vitro* cellular Pol activity assay using UVB-exposed NHEK cell extract.

Interestingly, NHEK Pol activity decreased at 200 mJ/cm^2 because most cells had died (data not shown).

Next, we investigated the effect of incubation time on Pol activity following UVB irradiation. Pol activity was highest among NHEK incubated for 4 h (100 mJ/cm^2), among cells cultured 2–24 h, suggesting that UVB-damaged DNA repair activity peaks 4 h after irradiation (Figure 2B). These results suggest that the culture conditions most suitable for increasing Pol activity is 150 mJ/cm^2 of UVB irradiation, and incubation for 4 h. Next, we screened Pol enhancer activity from tropical plant extracts using these same conditions.

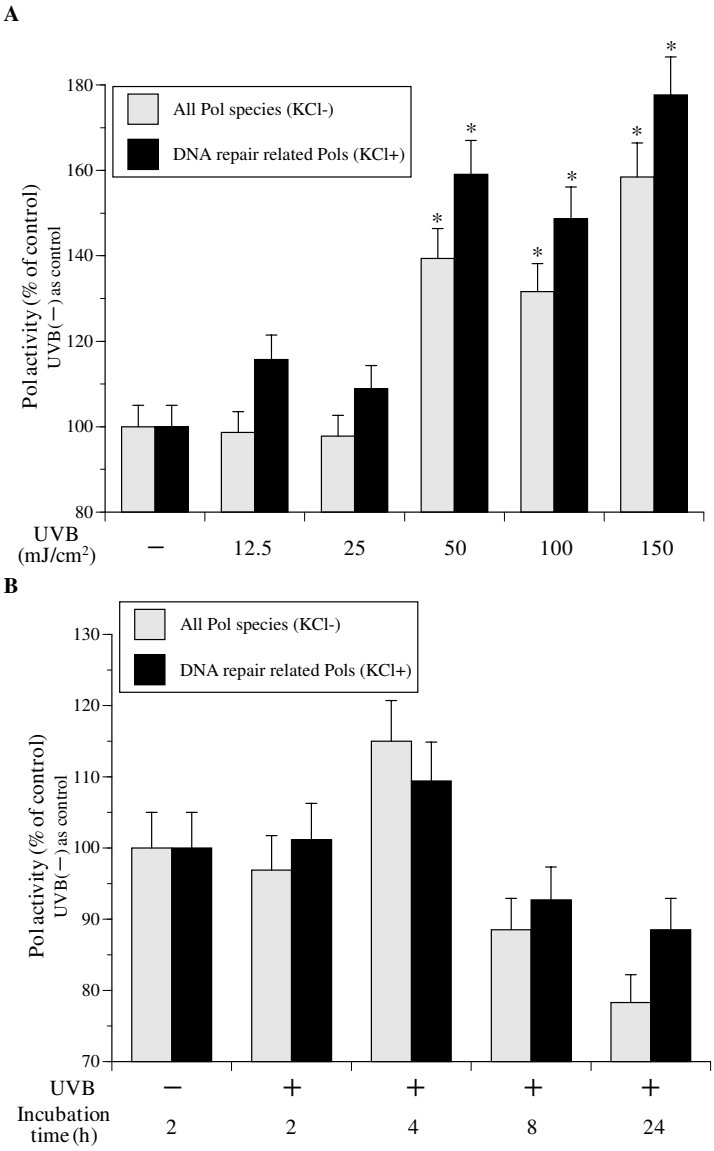

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Figure 2. Examination of cellular Pol activity in UVB-exposed NHEK. (A) Cellular Pol activity was dependent upon the level of UVB irradiation (0–150 mJ/cm²). NHEK were cultured for 1 h after UVB irradiation. (B) Pol activity is dependent upon incubation time (2–24 h) above 100 mJ/cm² UVB-exposed NHEK. Gray bars and black bars are human all Pols and DNA repair-related Pol species, respectively (the standard reaction conditions without or with 120 mM KCl, respectively). The Pol activity of vehicle control without UVB irradiation was taken as 100%. All data are expressed as mean ± SEM (n = 3). *$P < 0.05$ compared with the UVB (−) vehicle control.

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3. Screening for plant Pol activity enhancers in UVB-irradiated NHEK

3.1. Screening tropical plant extracts

We screened for UVB-induced Pol active compounds, testing 80% ethanol extracts from 50 tropical plants. The Rose Myrtle extract was the strongest stimulator of Pol activity in UVB-exposed NHEK among the plant extracts tested. Rose Myrtle is a shrub of the Myrtaceae family, originating from Southeast Asia. It grows under various conditions and is an invasive species in some areas where it was introduced as an ornamental plant. Parts of this plant (leaves, roots, buds, and fruits) have long been used in traditional Vietnamese, Chinese, and Malay medicine. In particular, the fruits have been used to treat diarrhea and dysentery, and to boost the immune system [24]. Rose Myrtle fruit has an astringent taste and a deep purple color at maturity. All these properties may be explained, at least partially, by the presence of polyphenols.

3.2. Isolation of the bioactive compound from Rose Myrtle fruit

Rose Myrtle fruit was obtained from Maechu Co. Ltd. (Nara, Japan) and Shinwa Bussan Co., Ltd. (Osaka, Japan) and 100 g extracted with 1 L of 80% ethanol. The evaporated extract (6.6 g) was dissolved in distilled water, and subjected to hydrophobic column chromatography (Diaion HP-20; Sigma Aldrich, St. Louis, MO, USA). Three hydrophobic chromatography fractions were collected: water, methanol, and acetone. The methanol fraction was evaporated (2.6 g) and subjected to silica gel 60 column chromatography and eluted with chloroform: methanol: water (v:v:v, 10:5:1). The active fraction was obtained and purified by reverse-phase silica gel column chromatography (Chromatorex ODS DM1020T; Fuji Silysia Ltd, Durham, NC, USA), and continuous high-performance liquid chromatography. This process resulted in a white powdery compound **1** (1.14 mg).

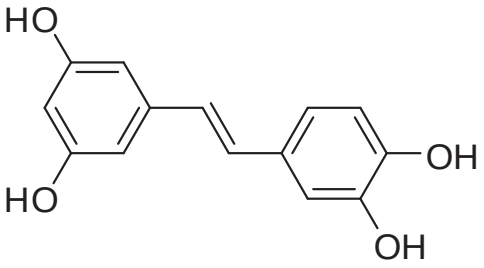

Figure 3. Structure of purified compound **1** (piceatannol) from Rose Myrtle fruit (*Rhodomyrtus tomentosa*).

Compound **1** was identified as the polyphenol piceatannol using high-resolution mass spectrometry and ^{1}H and ^{13}C nuclear magnetic resonance (NMR) data (Figure 3), consistent with previously published spectroscopic data [25]. Therefore, we used purified piceatannol

(98%) as determined by NMR analysis (data not shown). Additionally, we further investigated whether Rose Myrtle extract and piceatannol stimulated cellular Pol activity, blocked UVB-induced cell damage, and suppressed the inflammatory mediator prostaglandin E_2 (PGE_2) in NHEK.

4. Effect of Rose Myrtle fruit extract and piceatannol on Pol activity in UVB-exposed NHEK

The effect of purified piceatannol on Pol activity in cultured NHEK was investigated using the Pol enhancement assay (Figure 1). All Pol species are active in buffer with salts such as NaCl and KCl, whereas Pols α, δ, and ε are inhibited by salt [1] (Table 1). Therefore, the standard reaction mixture with or without 120 mM KCl was used to detect all Pol activity (Figure 4A) or DNA repair-related Pol activity (Figure 4B), respectively. The activity of DNA repair-related Pol species such as X and Y family Pols are enhanced by salt (120 mM KCl) [1]. The activities of purified calf Pol α and rat Pol β, which are B- and X-family Pols, respectively, were one-tenth lower and 1.5-fold higher with 120 mM KCl than those without KCl (data not shown). The ratios of cellular Pol activity in the standard reaction mixture without salt were higher than those with salt (Figure 4).

NHEK Pol activity with or without UVB irradiation and test compound treatment was similar (Figure 4). In non-treated compounds, UVB exposure at 100 mJ/cm^2 resulted in an approximately 1.2-fold increased Pol activity. In non-UVB-irradiated NHEK, extract and piceatannol enhanced NHEK Pol activity slightly. Moreover, Pol activities were raised significantly by the extract and piceatannol treatment in UVB-exposed NHEK. These results indicate a synergistic effect of UVB irradiation and Rose Myrtle extract and/or piceatannol on the induction of Pol enzyme activity, particularly DNA repair-related Pols.

5. Effect of the Rose Myrtle fruit extract and piceatannol on UVB-exposed NHEK cell viability

We next sought to investigate whether piceatannol inhibited NHEK proliferation. NHEK were grown to sub-confluence in KGM in 48-well plates, washed with Hank's buffer, irradiated with UVB (50 mJ/cm^2), and treated with test compound for 24 h in KGM. After treatment, cell viability (percent living cells) was evaluated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay [26].

Treatment of cultured NHEK with Rose Myrtle fruit extract and isolated piceatannol at concentrations up to 100 and 20 μg/mL, respectively, did not induce cytotoxic effects (cell viability >95% after 24 h treatment, data not shown). Therefore, the following experiments were conducted within the concentration range mentioned above. NHEK were treated after UVB irradiation (50 mJ/cm^2), and cell viability examined 24 h post-irradiation and compared

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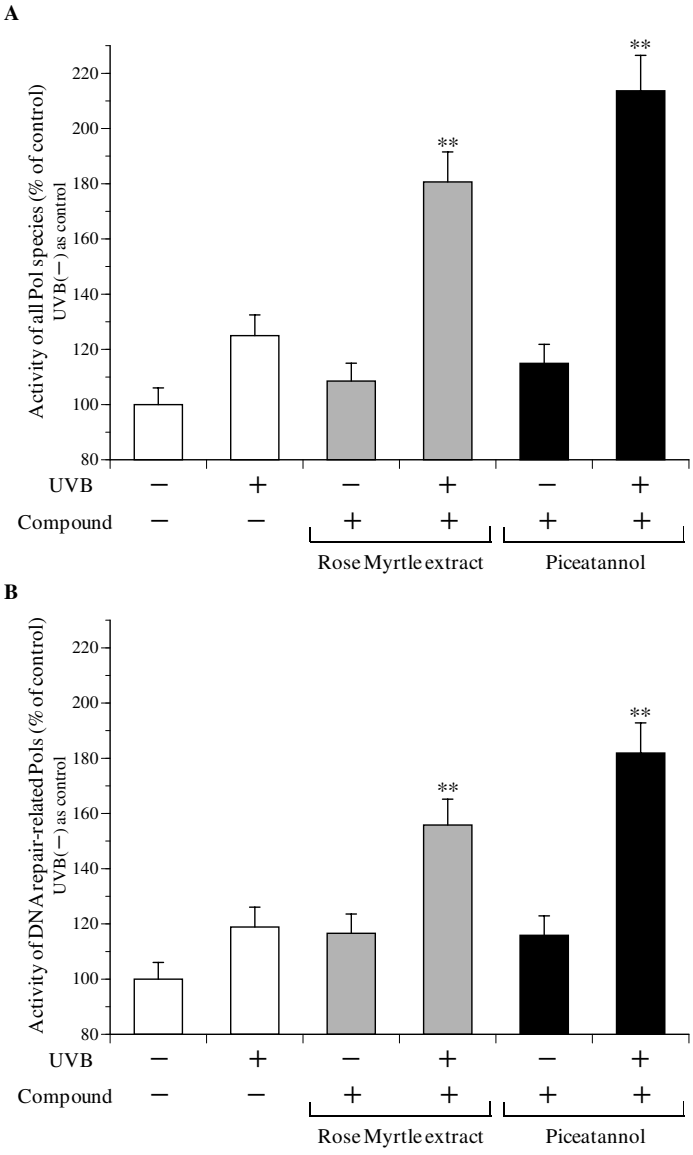

Figure 4. Effect of Rose Myrtle extract and piceatannol on NHEK Pol activity with or without UVB irradiation. (A) Activity of human all Pols species under standard reaction conditions without KCl. (B) Activity of DNA repair-related Pol species under standard reaction conditions with 120 mM KCl. NHEK were incubated for 24 h with or without each compound (10 μg/mL Rose Myrtle extract and 2 μg/mL piceatannol) before UVB (100 mJ/cm^2) irradiation. Pol activity of vehicle control without UVB irradiation was taken as 100%. All data are expressed as mean ± SEM (n = 3). $^{**}P < 0.01$ compared with the UVB (−) vehicle control.

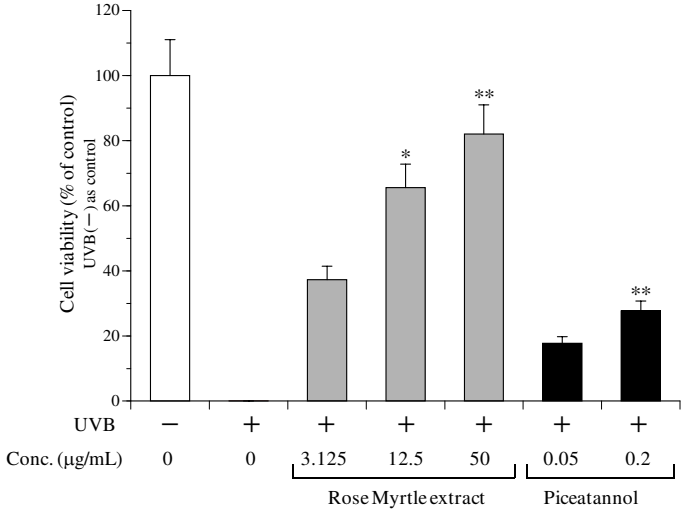

Figure 5. Effect of Rose Myrtle extract and piceatannol on cell viability of UVB-exposed NHEK. NHEK were irradiated with UVB (50 mJ/cm^2), and treated with each compound at the indicated concentrations. MTT assays were used to evaluate living cells 24 h after treatment. Cell viability of vehicle control with or without UVB irradiation was taken as 0% or 100%, respectively. All data are expressed as mean ± SEM (n = 6). *P < 0.05 and **P < 0.01 compared with the UVB (−) vehicle control.

with non-treated cells. Extract markedly inhibited UVB-induced NHEK cytotoxicity in a dose-dependent manner (Figure 5). Cell viability with 50 μg/mL of extract increased to more than 80% in non-treated cells. Piceatannol also increased UVB-exposed NHEK cell viability in a dose-dependent manner, suggesting it is the protective component of Rose Myrtle extract. At the same time, piceatannol-4'-*O*-β-D-glucopyranoside, a glucoside form of piceatannol, did not exhibit a protective effect (data not shown), suggesting that the aglycone structure is important for protective activity.

6. Effect of Rose Myrtle fruit extract and piceatannol on CPD production in UVB-exposed NHEK

CPD formation is an important product of DNA damage and mutagenesis [27]. We investigated the hypothesis that Rose Myrtle extract and its polyphenolic component, piceatannol, may influence the removal of CPD from DNA in UVB-irradiated NHEK. To measure CPD production, NHEK were grown to sub-confluence using KGM in 60-mm^2 culture dishes, and treated with test compound for 24 h. Cultures were then washed with Hank's buffer, irradiated with UVB (80 mJ/cm^2), and treated with test compound for 6 h in KGM. Cultured cells were collected by a cell scraper after treatment and nuclear DNA purified using a QIAamp Blood Kit (Qiagen, Tokyo, Japan). CPD levels in the quantified DNA were measured by enzyme-

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linked immunosorbent assay (ELISA) using an anti-CPD monoclonal antibody (Cosmobio Co. Ltd., Tokyo, Japan), according to the manufacturer's protocol.

Exposure of NHEK (80 mJ/cm^2 UVB) induced CPD formation as measured immediately after irradiation, and served as a reference for DNA damage (Figure 6). To evaluate DNA repair in irradiated cultures, CPD levels were measured after UVB exposure and compared with the non-repaired reference. Both Rose Myrtle extract and piceatannol decreased CPD production in UVB-exposed NHEK in a dose-dependent manner, with 10 μg/mL of extract and 0.5 and 2 μg/mL of piceatannol exhibiting 20% CPD reduction compared with non-treated control cells. These results suggest that Rose Myrtle extract and/or piceatannol might have DNA repair activity against UVB-damaged DNA in NHEK. As shown in Figure 4, both Rose Myrtle extract and piceatannol increased cellular Pol activity in NHEK, suggesting that activation of these enzymes, in particular DNA repair-related Pols β and η, contributes to reduced CPD production.

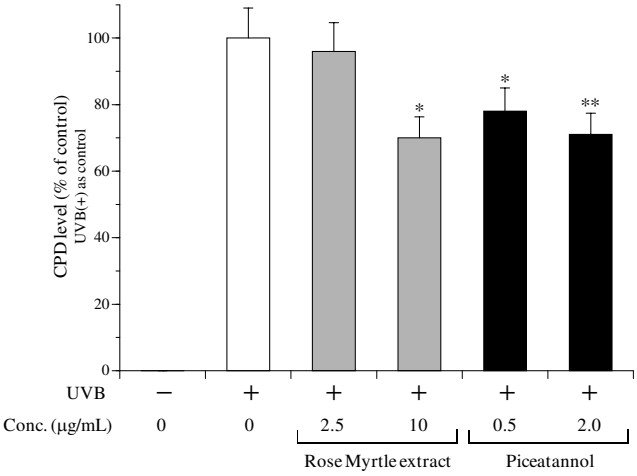

Figure 6. Effect of Rose Myrtle extract and piceatannol on UVB-induced CPD production in NHEK. NHEK were incubated with each compound at the indicated concentrations before and after UVB (80 mJ/cm^2) irradiation. CPD was quantitatively evaluated by DNA-ELISA. CPD production by vehicle control with or without UVB irradiation was taken as 100% or 0%, respectively. All data are expressed as mean ± SEM (n = 6). *$P < 0.05$ and **$P < 0.01$ compared with the UVB (+) vehicle control.

7. Effect of Rose Myrtle fruit extract and piceatannol on PGE_2 production in UVB-exposed NHEK

We next examined the possible influence of extract and piceatannol on PGE_2 production to examine whether they are associated with anti-inflammatory properties in NHEK. To measure

PGE_2 production, NHEK were grown to sub-confluence in KGM using 48-well plates. Cells were then cultured in KGM without hydrocortisone for 1 day, irradiated in Hank's buffer with UVB (50 mJ/cm^2), and treated with test compound for 24 h in KGM without hydrocortisone. After treatment, the culture medium was collected and centrifuged. Supernatant PGE_2 was analyzed using PGE_2 EIA Kits (Cayman Chemical Co., Ann Arbor, MI, USA).

UVB irradiation increased PGE_2 secretion by approximately 2.9-fold in non-irradiated NHEK to 238.6 pg/mL, and this amount was set to 100% as a positive control (Figure 7). Both extract and piceatannol lead to decreased PGE_2 production in a dose-dependent manner, implying that Rose Myrtle extract and/or piceatannol suppress UVB-stimulated inflammation.

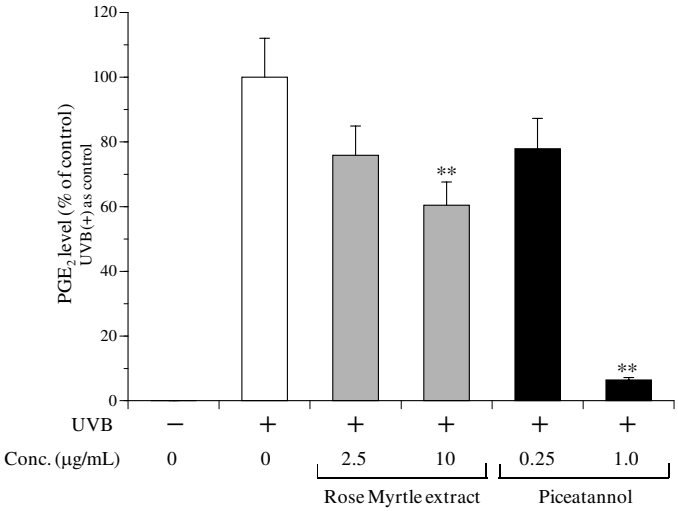

Figure 7. Effect of Rose Myrtle extract and piceatannol on UVB-induced PGE_2 production in NHEK. NHEK were incubated with each compound at the indicated concentrations after UVB (50 mJ/cm^2) irradiation. Supernatant PGE_2 was quantitatively evaluated by ELISA. PGE_2 production by vehicle control with or without UVB irradiation was taken as 100% or 0%, respectively. All data are expressed as mean ± SEM (n = 5). $^{**}P < 0.01$ compared with the UVB (+) vehicle control.

8. Discussion

We established an *in vitro* Pol activator assay using cell extracts from UVB-exposed NHEK (Figures 1 and 2) to demonstrate cellular Pol enhancement in 80% ethanol extracts from Rose Myrtle fruit and its key active ingredient, piceatannol (Figure 3). About 90% of skin inflammation cases are attributed to solar UV radiation, particularly its UVB component, which is absorbed efficiently by cellular DNA [28]. UVB radiation penetrates the skin epidermis, inducing both direct and indirect DNA-damaging effects. Rose Myrtle extract and piceatannol

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increased cell viability in UVB-exposed NHEK (Figure 5), and promoted removal of CPD photoproducts (Figure 6), suggesting an improvement in DNA damage repair. The formation of CPD and 6-4 pyrimidine-pyrimidone photoproducts are the most predominant DNA lesions in skin after UVB and UVA exposure [27, 29]. The main repair mechanism of UVB-induced DNA damage is nucleotide excision repair (NER). When skin cells are exposed to excessive UV radiation, NER capacity is reduced and CPD lesions remain, resulting in cell death, senescence, mutagenesis, and carcinogenesis of the skin [29]. Presumably, enhancement of DNA repair is one of the reasons why extract and piceatannol exert a protective effect on UVB-irradiated NHEK viability, and on sun-damaged cell formation in UVB-irradiated human skin explants.

We also analyzed the effect of Rose Myrtle extract and piceatannol on *in vitro* Pol activity in UVB-irradiated cultured NHEK cell extracts, which significantly enhanced enzyme activity (Figure 4). Pols synthesize DNA and have an essential role in genome duplication, but are also crucial for protecting cells against the effects of DNA damage. In both normal and cancerous cells, DNA is subjected to damage from many sources. Water-catalyzed reactions and attack by reactive oxygen species inflict continual damage. Ubiquitous sources of lesions include naturally occurring ionizing radiation, such as UV radiation from the sun. The toxic and mutagenic consequences of such damage are minimized by distinct DNA repair pathways, including BER and NER. These repair mechanisms rely on a Pol to fill gaps in the DNA that are left by the removal of damaged bases. If DNA damage is unrepaired, cells often tolerate it by TLS during DNA replication to insert a base opposite a lesion and bypass the damage. Finally, when breaks and gaps arise in DNA they are repaired by various mechanisms, including homologous recombination and various non-homologous end-joining processes. Pols are also essential components of these pathways.

There are 15 different Pols encoded in mammalian genomes, which are specialized for replication, repair, or tolerance of DNA damage (Table 1). New evidence is emerging for lesion-specific and tissue-specific functions of Pols [5]. The most notable TLS Pol for the bypass of UV radiation-induced DNA damage is Pol η. Currently, Pol η is the only Pol for which a deficiency is known to predispose humans to cancer [30]. The inherited disorder xeroderma pigmentosum (XP) is associated with a greatly increased risk of sunlight-induced carcinomas of the skin, and individuals with the variant type of the condition, XP-V, have disabling mutations in Pol η [30, 31]. $Polh^{-/-}$ mice mirror the XP-V phenotype as they rapidly develop UV radiation-induced tumors. $Polh^{+/-}$ mice are also susceptible to UV radiation-induced skin carcinogenesis, although at a lesser rate [32]. UV irradiation of XP-V cells cause DNA double-strand breaks owing to the absence of Pol η TLS function, which causes DNA replication forks to stall and collapse at sites of DNA damage on the template strand [33]. Prolonged replication delays in the absence of Pol η may also inhibit DNA repair of UV radiation-induced lesions [34]. Unirradiated $POLH^{-/-}$ cells have more chromatid breaks than normal, including breaks at a common fragile site [35]. Patients with XP-V have an increased incidence of squamous cell carcinoma of the skin, and experiments have been conducted to determine whether *POLH* mutations are associated with sporadic skin carcinomas [36] or other human cancers. However, no mutations affecting Pol η function have yet been identified.

We have demonstrated that Rose Myrtle extract and piceatannol decrease UVB-induced secretion of the inflammatory mediator PGE_2 (Figure 7). Notably, NHEK-derived inflammatory mediators play an important role in the development of the inflammatory reaction in UVB-exposed skin [37]. Numerous studies have demonstrated that PGE_2 mediates induction of erythema, angiogenesis, vasodilatation, and vascular permeability [38], and PGE_2 signaling pathways promote photoaging and development of UVB-induced skin carcinogenesis [39]. Taken together, the inhibitory effects of Rose Myrtle extract and piceatannol against UVB-induced PGE_2 expression in NHEK demonstrate the anti-inflammatory properties of these compounds. This observation supports the idea that these compounds have anti-inflammatory capability not only against UVB-induced inflammation, but also against inflammatory reactions caused by other irritants. Thus, there appears to be a relationship between enhanced cellular Pol activity in UVB-irradiated NHEK and reduced PGE_2 anti-inflammation, but further investigation will be required to support this hypothesis. Because both Rose Myrtle extract and piceatannol do not absorb UVB light, we suggest that they act in a non-sunlight dependent manner to protect against UVB-induced inflammatory induction.

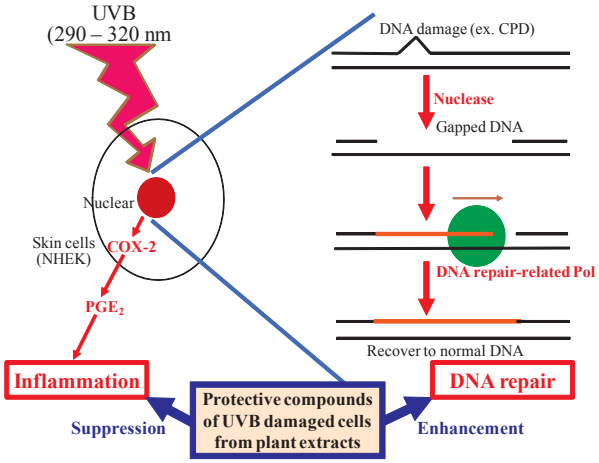

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Figure 8. The relationship between enhanced DNA repair-related Pol activity and suppression of inflammation in UVB-irradiated skin cells treated with plant extract.

9. Conclusion

Rose Myrtle fruit extracts were the most effective among 50 tropical plants at increasing cell viability in UVB-irradiated NHEK. Rose Myrtle fruit extract and its isolated polyphenolic component, piceatannol, were found to decrease production of CPD and PGE_2, a DNA damage photoproduct and an inflammatory mediator, respectively. These results suggest that Rose

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Myrtle piceatannol protects skin from UVB-induced damage via enhancement of DNA repair-related Pol enzyme activity and suppresses inflammation. We demonstrate the utility of an *in vitro* Pol activity screening method using UVB-irradiated NHEK (Figure 1), show that it is easy to perform, and provides rapid results. The selected plant extract Pol activity enhancement compounds (Figure 8), may have potential as non-sunscreen cosmetics.

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Author details

Sawako Shiratake[1], Takefumi Onodera[2,3], Yuka Sakamoto[3], Tatsuo Nakahara[1], Hiroyasu Iwahashi[1] and Yoshiyuki Mizushina[2,4*]

*Address all correspondence to: mizushina@shinshu-u.ac.jp

1 Research Center, Maruzen Pharmaceuticals Co., Ltd., Onomichi, Hiroshima, Japan

2 Cooperative Research Center of Life Sciences, Kobe Gakuin University, Chuo-ku, Kobe, Hyogo, Japan

3 Laboratory of Food and Nutritional Sciences, Faculty of Nutrition, Kobe Gakuin University, Nishi-ku, Kobe, Hyogo, Japan

4 Graduate School of Agriculture, Shinshu University, Minamiminowa-mura, Kamiina-gun, Nagano , Japan

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References

[1] Kornberg A, Baker TA. Eukaryotic DNA replication. In: Freeman WD and Co. (Ed.) DNA replication 2nd edition. University Science Books, California; 1992. Chapter 6, p197-225.

[2] Hubscher U, Maga G, Spadari S. Eukaryotic DNA polymerases. Annual Review of Biochemistry 2002;71 133-163.

[3] Bebenek K, Kunkel TA. In: Yang W. (ed.) DNA Repair and Replication. Advances in Protein Chemistry, San Diego, Elsevier; 2004. p137-165.

[4] Loeb LA, Monnat Jr. RJ. DNA polymerases and human disease. Nature Reviews Genetics 2008;9: 594-604.

[5] Lange SS, Takata K, Wood RD. DNA polymerases and cancer. Nature Reviews Cancer 2011;11 96-110.

[6] Johnson RE, Washington MT, Prakash S, Prakash L. Fidelity of human DNA polymerase η. The Journal of Biological Chemistry 2000;275 7447-7450.

[7] Servant L, Cazaux C, Bieth A, Iwai S, Hanaoka F, Hoffmann JS. A role for DNA polymerase β in mutagenic UV lesion bypass. The Journal of Biological Chemistry 2002;277 50046-50053.

[8] Berneburg M, Plettenberg H, Krutmann J. Photoaging of human skin. Photodermatology, Photoimmunology and Photomedicine 2000;16 239-244.

[9] Verschooten L, Claerhout S, Van Laethem A, Agostinis P, Garmyn M. New strategies of photoprotection. Photochemistry and Photobiology 2006;82 1016-1023.

[10] Moriwaki S, Takahashi Y. Photoaging and DNA repair. Journal of Dermatological Science 2008;50 169-176.

[11] Kripke ML, Cox PA, Alas LG, Yarosh DB. Pyrimidine dimers in DNA initiate systemic immunosuppression in UV-irradiated mice. Proceedings of the National Academy of Sciences of the United States of America 1992;89 7516-7520.

[12] Autier P. Sunscreen abuse for intentional sun exposure. British Journal of Dermatology 2009;161 40-45.

[13] Schroeder P, Krutmann J. What is need for a sunscreen to provide complete protection. Skin Therapy Letter 2010;15 4-5.

[14] Matsui MS, Hsia A, Miller JD, Hanneman K, Scull H, Cooper KD, Baron E. Non-sunscreen photoprotection: antioxidants add value to a sunscreen. Journal of Investigative Dermatology Symposium Proceedings 2009;14 56-59.

[15] Yaar M, Gilchrest BA. Photoageing: mechanism, prevention and therapy. British Journal of Dermatology 2007;157 874-887.

[16] Nichols JA, Katiyar SK. Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. Archives of Dermatological Research 2010;302 71-83.

[17] Sakaguchi K, Sugawara F, Mizushina Y. Inhibitors of eukaryotic DNA polymerases. Seikagaku 2002;74 244-251.

[18] Mizushina Y. Specific inhibitors of mammalian DNA polymerase species. Bioscience, Biotechnology, and Biochemistry 2009;73 1239-1251.

[19] Mizushina Y. Screening of novel bioactive compounds from food components and nutrients. Journal of Japanese Society of Nutrition and Food Science 2011;64 377-384.

[20] Mizushina Y, Yagi H, Tanaka N, Kurosawa T, Seto H, Katsumi K, Onoue M, Ishida H, Iseki A, Nara T, Morohashi K, Horie T, Onomura Y, Narusawa M, Aoyagi N, Takami K, Yamaoka M, Inoue Y, Matsukage A, Yoshida S, Sakaguchi K. Screening of inhibitor of eukaryotic DNA polymerases produced by microorganisms. Journal of Antibiotics (Tokyo) 1996;49 491-492.

[21] Mizushina Y, Tanaka N, Yagi H, Kurosawa T, Onoue M, Seto H, Horie T, Aoyagi N, Yamaoka M, Matsukage A, Yoshida S, Sakaguchi K. Fatty acids selectively inhibit eukaryotic DNA polymerase activities in vitro. Biochimica et Biophysica Acta 1996;1308 256-262.

[22] Mizushina Y, Yoshida S, Matsukage A, Sakaguchi K. The inhibitory action of fatty acids on DNA polymerase β. Biochimica et Biophysica Acta 1997;1336 509-521.

[23] Lindell TJ, Weinberg F, Morris PW, Roeder RG, Rutter WJ. Specific inhibition of nuclear RNA polymerase II by α-amanitin. Science 1970;170 447-449.

[24] Do TL. SIM. In Medicine plants and remedies of Vietnam (16th ed.). Hanoi, Vietnam: Thoi Dai Publication House. 2011, p434-435.

[25] Kashiwada Y, Nonaka G, Nishioka I, Nishizawa M, Yamagishi T. Studies on Rhubarb (*Rhei Rhizoma*). XIV. : Isolation and Characterization of Stilbene Glucosides from Chinese Rhubarb. Chemical and Pharmaceutical Bulletin 1988;36 1545-1549.

[26] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods 1983;65 55-63.

[27] Marrot L, Meunier JR. Skin DNA photodamage and its biological consequences. Journal of the American Academy of Dermatology 2008;58 S139-148.

[28] Gailani MR, Leffell DJ, Ziegler A, Gross EG, Brash DE, Bale AE. Relationship between sunlight exposure and a key genetic alteration in basal cell carcinoma. The Journal of the National Cancer Institute 1996;88 349 -354

[29] Pagès V, Fuchs RP. How DNA lesions are turned into mutations within cells? Oncogene 2002;21 8957-8966.

[30] Masutani C, Araki M, Yamada A, Kusumoto R, Nogimori T, Maekawa T, Iwai S, Hanaoka F. Xeroderma pigmentosum variant (XP-V) correcting protein from HeLa cells has a thymine dimer bypass DNA polymerase activity. EMBO Journal 1999;18 3491-3501.

[31] Inui H1, Oh KS, Nadem C, Ueda T, Khan SG, Metin A, Gozukara E, Emmert S, Slor H, Busch DB, Baker CC, DiGiovanna JJ, Tamura D, Seitz CS, Gratchev A, Wu WH,

Chung KY, Chung HJ, Azizi E, Woodgate R, Schneider TD, Kraemer KH. Xeroderma pigmentosum-variant patients from America, Europe, and Asia. Journal of Investigative Dermatology 2008;128 2055-2068.

[32] Lin Q, Clark AB, McCulloch SD, Yuan T, Bronson RT, Kunkel TA, Kucherlapati R. Increased susceptibility to UV-induced skin carcinogenesis in polymerase eta-deficient mice. Cancer Research 2006;66 87-94.

[33] Limoli CL, Giedzinski E, Bonner WM, Cleaver JE. UV-induced replication arrest in the xeroderma pigmentosum variant leads to DNA double-strand breaks, γ-H2AX formation, and Mre11 relocalization. Proceedings of the National Academy of Sciences of the United States of America 2002;99 233-238.

[34] Auclair Y, Rouget R, Belisle JM, Costantino S, Drobetsky EA. Requirement for functional DNA polymerase eta in genome-wide repair of UV-induced DNA damage during S phase. DNA Repair (Amst) 2010;9 754-764.

[35] Rey, L. et al. Human DNA polymerase eta is required for common fragile site stability during unperturbed DNA replication. Molecular and Cellular Biology 2009;29 3344-3354.

[36] Glick E, White LM, Elliott NA, Berg D, Kiviat NB, Loeb LA. Mutations in DNA polymerase eta are not detected in squamous cell carcinoma of the skin. International Journal of Cancer 2006;119 2225-2227.

[37] Kabashima K, Nagamachi M, Honda T, Nishigori C, Miyachi Y, Tokura Y, Narumiya S. Prostaglandin E2 is required for ultraviolet B-induced skin inflammation via EP2 and EP4 receptors. Laboratory Investigation 2007;87 49-55.

[38] Trompezinski S, Pernet I, Schmitt D, Viac J. UV radiation and prostaglandin E2 upregulate vascular endothelial growth factor (VEGF) in cultured human fibroblasts. Inflammation Research 2001;50 422-427.

[39] Rundhaug JE, Miculec C, Pavone A, Fisher SM. A role for cyclooxygenases-2 in ultraviolet light-induced skin carcinogenesis. Molecular Carcinogenesis 2007;46 692-698.