Identification of cellular mechanisms protecting against UVB induced damage in vitro and in vivo

OTKA K120206 Closing report

INTRODUCTION

Ultraviolet radiation (UV) is part of the electromagnetic spectrum and a component of sunlight. It is divided into three wavelength ranges. UVC (100-280 nm) is completely absorbed by the atmosphere and has no clinical relevance. Human skin is exposed to UVA (315-400 nm, 95%) and UVB (280-315 nm, 5%). UV radiation has some beneficial effects on human health like with Vitamin D synthesis, mood improvement, antimicrobial peptide synthesis, but it is also the main environmental stressor and carcinogen of the human skin. Acute irradiation of UVB can result in sunburn, phototoxic reactions and immunosuppression. Long-lasting chronic UVB is the main risk factor for the most common human malignancies, non-melanoma skin cancers (squamous cell carcinoma [SCC], and basal cell carcinoma [BCC]) and it also contributes to melanoma development(1). The fact that over 60% of all human malignancies in Caucasians are caused by UV radiation and its incidence is increasing(2) highlights the importance of better understanding the effect of UV light on the skin. Chronic UVA is the most important factor responsible acute phototxicity together with environmental factors (like drugs, plant extracts)(3) and in chronic skin changes for premature skin aging called photoaging. Its role in photocarcinogenesis has also been shown, although, UVB has been proven to be the major photocarcinogen(4).

The biological effects of UVB radiation are mostly attributed to direct DNA damage, formation of **cyclobutane pyrimidine dimers** (CPDs), 6-4 photoproducts and its derivatives. Unrepaired CPDs are mainly responsible for the cytotoxic and mutagenic effects of UVB radiation(5). The removal of CPDs from the mammalian genome takes place by **nucleotide excision repair** (NER) which is slow and incomplete(6). NER is a multistep repair mechanism involving more than 30 proteins. If NER is ineffective, or the number of CPDs exceed its repair capacity, persisting CPDs induce cell cycle arrest in order to prevent the multiplication of mutated chromosomes, and may lead to induction of apoptosis, however, genomic instability or skin carcinogenesis may also result from unrepaired CPDs(7, 8). The central molecule orchestrating these events is **p53 tumorsuppressor protein(9).** It accumulates in detectable amounts by immune histochemistry(10) after acute UVB irradiation and also when mutated(9) frequently baring UV signature mutations(11). Accumulation of mutated p53 is a good marker for microscopic precancers(12) and **field carcinogenesis** where large areas of the entire sun exposed skin contain oncogene and tumorsuppressor gene mutations, as most commonly seen in **organ transplant recipients (OTR)**(13, 14). Besides NER, which is the only CPD removal mechanism in placental mammals, all other phylogenetically less complex living organism have a more efficient mechanism to repair CPDs via **photoreactivation**. Photoreactivation is a light-dependent DNA repair performed by **photolyases**(15). These DNA repair proteins are structure-specific, bind to pyrimidine dimers (CPDs or 6-4PPs) and convert them directly back to the original structure using the energy of visible light(16, 17). All known photolyases rapidly remove UV-induced DNA lesions keeping the genomic integrity in a light-dependent cyclic redox reaction(18). Viral transfection of cDNA of *Potorous tridactylus* CPD photolyase into mammalian cells was published to be able to correct rapidly UVB induced CPDs(19). Earlier studies showed the topically applied photolyase protein is capable of protecting the skin from UVB damage, but the nuclear localization of the protein was not shown and photoabsorption properties of the protein itself was not excluded(20). A recent paper using special formulation of photolyase applied it after UVB irradiation and showed promising clinical response in humans(21, 22) but with a few experimental evidenceses.

It is widely accepted that UVB can also initiate free radicals mainly oxidative damage (**reactive oxygen species (ROS)**)(23, 24). There is some data on immediate ROS reaction(25) mediated by intracellular Ca^{2+} (26, 27), but more data support production due to enzymatic activity and mitochondrial electron transfer(28). Enzymes and small molecules scavenging free radicals are responsible for cellular defense against ROS. Excessive production or decreased elimination of ROS causes cell death or mutations and has been shown to contribute to carcinogenesis(29, 30). Recently our knowledge of the important messenger and housekeeping function(31, 32) of ROS has expanded and ROS has been proven to play a role in redox

regulation of stress signaling, apoptosis and senescence(33, 34). It may also contribute to CPD formation because in the presence of antioxidants number of CPDs were reduced(35).
There are several other players which modify in cellular effects of UV. Among them **Poly (ADP-ribose) polymerase-1** (PARP-1), which plays a role in chromatin structure, inflammation, cell cycle, cell death, calcium homeostasis, metabolic regulation and also DNA repair(36). Recent data support the role of PARP-1 in NER(37), DNA damage recognition and basic cellular functions(38). To understand some of these processes members of our research group have identified the role of polyunsaturated fatty acids signaling in the proinflammatory effect of PARP(39). We also learned that UVB markedly affects lipid transporters in keratinocytes contributing to UV induced barrier disruption(40) and alters purinergic receptor signaling in keratinocytes(41). We also identified the effect of zinc homeostasis in UV response(42) and earlier we determined the most reliable housekeeping genes for UV gene-expression experiments(43).
UVB has an even more complex effect on cells modulating most cell functions: DNA damage, induction of ROS, signal transduction, induction of cytokines [pro-inflammatory, immunosuppressive], apoptosis, senescence, and extracellular remodeling. Moreover, besides its direct cellular effects UV also acts via bystander mechanisms(44). All of these play some role in the carcinogenic effect of chronic UV irradiation. Complexity of UV carcinogenesis is well represented in OTR. Besides genetic susceptibility, high amount of sun exposure, fair skin type, the type and duration of transplantation, type of immunosuppressive drugs, immune status, certain HPV infections are predisposing factors for the development of skin cancers(45). Early detection and active treatment of the precancerous lesions and skin cancers are essential. Due to field cancerization, not only the visible lesions but the surrounding clinically normal appearing skin also needs to be treated to reduce the risk of new skin cancer formation. Therefore, field therapies have need to be applied(46). These include topical application of imiquimod, 5-fluorouracil, ingenol mebutate, and the use of **photodynamic therapy (PDT)(47, 48)** and **ablative lasers (CO2, Er:YAG**)(49, 50). Besides treatment, primary prevention and proper **sun protection** are the most important factors to avoid carcinogenesis(46). This is of paramount importance not only for OTR but also for the general population. Vast experimental, clinical and epidemiological data shows that skin cancer risk is reduced by broad band UV protection(51). Besides the important behavioral factors (clothing, avoiding sun exposure at highly sunny areas and time, seeking shades) topical sunscreens with different ingredients are widely used. These sunscreens mainly containing physical and chemical UV filters and have to be applied before the sun exposure(52). Recently there are several scientific publications showing the effectiveness of natural **plant derived molecules**, polyphenols(53) [flavonoids (catechins, isoflavones, proanthocyanidins anthocyanins) and non-flavonoid (phenolic acid, stilbene)] for topical(53, 54) and also systemic UV prevention(55, 56). These mainly act as antioxidants(57), but their other effects (anti-inflammatory, repair induction, anti-immunosuppressive) have also been shown(58, 59). The main pathways involved are: p38, COX, stat, MAPK/NFkb, ERK, Nrf2, IL12(60-62). Only few of the plant extracts have shown effectiveness when applied after the sun exposure. It is widely accepted that new strategies for sun protection are needed. The use of DNA repair enzymes (photolyase, T4 endonuclease), plant extracts, vitamins are considered topically and systemically(63-65). We have numerous methods to mitigate the effects of UV radiation before exposure, but to date no therapy is broadly available for efficient reduction of acute UV damage that could be applied after the skin has already been subjected to UV radiation. Despite our extensive knowledge about cellular UV response, there are still numerous unanswered questions. We seek to widen our knowledge about cellular mechanisms of UV response to identify feasible new post-UV exposure methods to reduce UV damage, and thus to help limit the morbidity caused by this important environmental carcinogen.

BACKGROUND IN OUR RESEARCH GROOUP

Although photolyase is a conceptually promising tool for use in human skin to eliminate UVB damage, its use is unlikely in the foreseeable future. Prior studies by others have used protein to eliminate CPDs but have failed to provide proof of the appropriate uptake of the protein into the cell nuclei. In our hands the *in vitro* synthetized CPD photolyase mRNA provided by Katalin Kariko (using the same methodology than in BionTeck/Pfizer COVID vaccine) proved to be a valuable tool *in vitro(66)* and helped us understand the mechanism of CPD based and non-CPD based changes after UVB.

However, we have not been able to successfully transfect photolyase mRNA in sufficient quantity into *in vivo* living skin, and no methods for efficient delivery of photolyase to intact epidermis are to date available. Nevertheless, the CPD photolyase mRNA *in vitro* model enabled us to identify genes regulated by CPD dependent and independent manner(67). However, many other important aspects of early UVB response that can be uncovered by this model are yet to be studied.

ORIGINAL AIMS OF THE PROPOSAL WERE:

I. Primary aim: to identify molecules and cellular mechanisms capable of ***reducing the consequences of acute UVB irradiation after UVB exposure*** and to establish their translational potential. We aim to identify ways to decrease and treat sunburn and mitigate its long term effects.

II. Secondary aims were:

1. To clarify the importance of CPD driven and independent cellular processes using *in vitro* synthesized pseudouridine CPD photolyase mRNA transfection into human cells
2. To determine the source and role of UVB induced ROS and to understand whether UVB induced ROS is produced directly due to UVB irradiation or it is mainly the consequence of the metabolic events initiated by UVB irradiation and mitochondrial changes and also PARP-1 mediated changes.
3. To determine the UV damage mitigating effect of a phyto-polyphenol compound *in vitro* on human keratinocytes when used after UV irradiation.
4. To assess the presence of CPDs in field-cancerized skin after UVB irradiation and estimate the clinical and molecular therapeutic effects of an ablative fractionated Er:YAG laser and PDT treatment on the precancerous skin.

III. Tertiary aims: To test the *in vivo* effectiveness of the promising phyto-polyphenol compound in decreasing UVB induced epidermal changes when the compound is applied only after UVB irradiation in a murine model. To conduct initial safety and efficacy studies of the compound in healthy human subjects and to establish the baseline ability of the compound to increase the minimal erythema dose when applied after UVB exposure.

LIMITATIONS

We were able to achieve most of our main goals, but partly due to the refutation of the basic concept of an objective, partly due to the problems caused by the closures due to the COVID pandemic (more difficult access to the laboratory and in vivo studies, patient involvement difficulties, equipment acquisition problems) we changed our original plan. We explored the background of the conceptual problem, and in part, we expanded our knowledge on the subject of UV carcinogenesis by performing epidemiological analyzes from our existing patient data.

REUSLTS:

BRIEFLY ACCORDING TO THE AIMS:

I. Primary aim: we identified molecular and cellular mechanisms capable of reducing the consequences of acute UVB irradiation before and also after UVB exposure.

II. Secondary aims:

1. We showed time course of the CPD removal on HaCaT and normal human keratinocytes and also that protective effect can be detected of pseudouridine containing CPD specific photolyase mRNA transfection even if the transfection occurred after the UVB irradiation.
2. UVB caused nuclear damage (CPDs) signal metabolic changes in cultured keratinocytes

2.1. We demonstrated the multifaceted role of poly-(ADP)- ribose polymerase-1 (PARP1) in UVB response. PARP1 inhibition via enhancing UVB-mediated DNA damage boosted energy metabolism and mitochondrial changes.

2.2. We also proved the role of mitochondria as the secondary source of UVB induced ROS in keratinocytes initiated by CPDs.

3. Plant extract and small molecules can inhibit nucleotide excision repair (NER), but their cellular biological effects can be unpredictable from their repair inhibitory effects.
3.1. We found no UVB-damage-mitigating effect of a phyto-polyphenol compounds *in vitro* on human keratinocytes when used before or after UV irradiation. Instead of protective effect of silymarin, we identified its strong phototoxicity under UVA irradiation.
3.2. We highlighted that small molecular inhibitors of NER instead of increasing UVB irradiated cellular DNA damage, unexpectedly they do not increase UVB induced mutation but opposite it even some of them reduce *in vitro* mutation burden of cells. Their effect on cell cycle delay, apoptosis, and autophagy may partly explain this finings.
4. Our team provided clinical and histopathological evidences that ablative fractionated Er:YAG laser improve the effect of photodynamic therapy (PDT) and we characterized the immune infiltration in PDT treated precancerous skin.
5. We conducted epidemiological studies on melanoma: these results suggest to screen melanomas among those over 65 years of age and that melanoma prevalence can be influenced by socioeconomic status. Serum S100B had a similar and independent prognostic strength in metastatic melanoma compared with serum LDH.
(III. Tertiary aims: Due to the phytophenol in vitro photosensitivity we have not performed any planed *ex vivo* or *in vivo* studies with these compounds. We even warn the scientific dermatological community about the phenomenon and careful usage of these plant extracts on human skin.)

DETAILED RESULTS:

1. **Time course of the CPD removal on HaCaT and normal human keratinocytes by photoreactivation showed significant protective effects on cell viability 6 hours after UVB irradiation. Pseudouridine containing CPD specific photolyase mRNA transfection effectively remove CPDs even if the transfection occurred after the UVB irradiation.**

In earlier studies, we transfected human keratinocytes with *in vitro* synthesized and nucleoside-modified mRNA encoding a marsupial CPD-specific photolyase. Using *in vitro* transcribed mRNAs superior translation and non-immunogenic nature were achieved by pseudouridine modification, codon-optimization, long polyA-tail, capping, and HPLC purification published by our foreign collaborative partner Katalin Karikó(68, 69) who provides photolyase mRNA for our studies. The mRNA synthesis based on similar methods used to design and synthesized COVID-19 BioNtech/Pfizer and Moderna vaccine. We achieved rapid and highly efficient repair of UVB-induced CPDs in transfected human keratinocytes exposed to low dose UVB and after UVB exposure immediate photoreactivating light, which resulted in significantly increased cell survival(66). Using cDNA microarray we analyzed CPD dependent and independent molecular events and found the involvement of JNK signaling pathway in a CPD dependent manner(67). By establishing this CPD dependent *in vitro* transcribed photolyase transfection system we created a new model uniquely capable of identifying and assessing direct DNA damage mediated and indirect UV induced cellular events. Using this model, we will study the time course effect of the CPD removal on the UVB induced apoptosis. The time available for eliminating UV induced DNA damage and improve acute UVB response is likely limited. Although knowing the actual time frame has obvious clinical implications it to date has never been established. In our prior work by removing CPDs immediately after UVB exposure, we observed 90% reduction in cell death (66). For therapeutic use, the time dependence of CPD removal on cell faith is crucial. Therefore, we established whether CPD removal later (4, 6 12 hours) after UVB irradiation is still capable of reducing UV induced changes, our aim was to identify the time threshold for HaCaT and NHEK keratinocyte. We found that *in vitro* synthesized CPD specific pseudouridine modified photolyase mRNA were able to restore cell viability within 6 hours after UVB irradiation of keratinocyte cultures. However, even 8 hours we have seen positive effects on cell viability(70). .

2. UVB caused nuclear damage (CPDs) signal metabolic changes in cultured keratinocytes

2.1. The multifaceted role of poly-(ADP) ribose polymerase-1 (PARP1) in UVB response was demonstrated. PARP1 inhibition via enhancing UVB-mediated DNA damage boosted energy metabolism and mitochondrial changes.

The acute and chronic UVB-mediated cellular responses are widely studied. However, little is known about the role **of poly (ADP-ribose) polymerase 1 (PARP1),** the earliest DNA damage responder protein in the regulation of mitochondrial function after UVB radiation. To elucidate the role of PARP1 in the UVB response, Veliparib (ABT-888), a widely used chemotherapeutic agent and PARP1 knockdown were used. We have detected the early activation of PARP1 after UVB which was completely suppressed by PARP inhibition (PARPi). We showed that loss of PARylation caused defective CPD repair. Furthermore, we observed a prolongation in G_2/M phase of cell cycle after PARP inhibition characteristic of severe DNA damage(71, 72). Interestingly apoptotic response after UVB could not be ameliorated by PARPi. Long-term survival of keratinocytes revealed that the combined treatment of UVB and PARP inhibition severely reduced cell proliferation. Interestingly, we detected that loss of PARylation decreased the frequency of mutations in UVB-treated cells (73) as evidenced by decreased number of colonies after ABT-888 treatment.

Accumulating evidence suggest that DNA damage can initiate mitochondrial biogenesis which is accompanied by the elevation in mitochondrial number, area, and mass(74). Transmission electron microscopic and confocal microscopic images revealed that UVB radiation triggered mitochondrial fusion and induced mitochondrial biogenesis, which culminated in enhanced mitochondrial activity and complex metabolic events which were more prominent in PARP-inhibited cells. Increased number of mitochondria, mitochondrial area and upregulation of proteins responsible for mitochondrial fusion were more robust after PARPi. mRNA expression of mitochondrial biogenesis regulators, increased mitochondrial cytochrome C oxidase I/ succinate dehydrogenase complex subunit A (MTCO1/SDHA) ratio, higher mitochondrial mass showed similar trends suggesting that elevated mitochondrial content is due to enhanced mitochondrial biogenesis and not defective mitochondrial turnover.

By measuring mitochondrial function, we observed that PARP inhibition enhanced UVB-mediated mitochondrial hyperpolarization, raised cellular ATP level and oxidative phosphorylation (OXPHOS). Boost in mitochondrial OXPHOS was dependent on ATM, AMPK, p53, AKT, and mTOR phosphorylation since inhibition of them lead to significant reduction in oxygen consumption after both UVB and PARPi. Moreover, enhanced mitochondrial activity seemed to induce a beneficial response after DNA damage, since decreasing mitochondrial biogenesis or ameliorating OXPHOS resulted in elevated cell death after UVB irradiation suggesting the protective effect of OXPHOS on cell viability.

Emerging evidence suggest that autophagy induction can be coupled to DNA damage response. Accordingly, UVB triggered the accumulation of autophagosomes which corroborates with enhanced LC3B expression. PARP inhibition resulted in enhanced autophagosome formation and higher LC3B protein level. Inhibition of the ATM, AMPK, p53, PI3K, and mTOR pathways caused significant reduction in LC3A/B and Parkin expression emphasizing their role in autophagy and mitophagy as well.

These results suggest the importance of PARP1 in the regulation of DNA damage response, autophagy and mitochondrial changes and contribute to the better understanding of PARP1 in the regulation of UVB response(75).

2.2. Mitochondria are the secondary source of UVB induced ROS in keratinocytes driven by CPDs in UVB exposed keratinocytes

We also investigated the **complex metabolic regulatory role of cyclobutene pyrimidine dimers** (CPD) in mediating the effects of UVB. CPDs are the main photoproducts of UVB. To study the CPD-elicited effects, we applied a previously developed model system (66, 76). In this model, CPD-photolyase mRNA transfection in human keratinocytes was used to ectopically overexpress photolyase, a photoreactive enzyme that efficiently and rapidly removes CPDs from the genome. Hence, we could identify the CPD-dependent and -independent effects of UVB. As expected from previous studies, UVB irradiation led to CPD accumulation, induced cell cycle blockade and cell death. CPDs were efficiently removed by photolyase and the photodamage was repaired over time in the whole genome. The UVB-induced decrease in cell cycle progression, keratinocyte proliferation, and viability were completely restored by photolyase activation, emphasizing the key role of CPDs in mediating cellular damage.

In parallel with DNA damage, we observed a decrease in NAD^+ level after UVB that was prevented by photolyase activation. We also detected the CPD-dependent activation of the NAD^+-consuming PARP1 protein and the Sirtuin enzyme family.

Mitochondrial morphology changes dynamically according to DNA damage. Confocal microscopy revealed that UVB dose-dependently induced mitochondrial fusion. This was supported by a decrease in the number of mitochondria, an increase in mitochondrial content, and an increase in the branching ability of mitochondria. If CPDs were removed by photoreactivation, no changes indicative of UVB-induced morphological rearrangement were detected. We observed an increase in the expression of mitochondrial fusion proteins and a CPD-dependent decrease in the expression of fission proteins. These results are consistent with the morphological alteration observed by confocal microscopy, suggesting that changes in protein expression underlies CPD-dependent mitochondrial fusion.

UVB-induced mitochondrial fusion was accompanied by mitochondrial biogenesis, as evidenced by enhanced Mitotracker Green accumulation inside the mitochondria. In addition, MTCO1/SDHA ratio was also increased. The intensity of Mitotracker Red CMXRos fluorescence indicating mitochondrial membrane hyperpolarization also increased. These changes were abrogated by the enzymatic removal of CPDs.

As far as functional changes, UVB increased the extracellular acidification rate (ECAR), indicating glycolysis, citrate synthase (CS) activity indicating Szent-Györgyi-Krebs cycle, and cellular basal oxygen consumption (OCR) indicating terminal oxidation. Thus, UVB induced a hypermetabolic state in keratinocytes via increased flux of core catabolic pathways. The enzymatic removal of CPDs prevented UVB-induced metabolic changes. We have previously shown that chemical inhibition of ATM, AMPK, p53, AKT, and mTOR proteins causes a significant decrease in the UVB-induced oxidative phosphorylation. Western blot analysis revealed that phosphoproteins expression was increased in a CPD-dependent manner. We demonstrated that the expression of the fatty acid transport and fatty acid oxidation regulators were all similarly increased in a CPD-dependent manner. These data suggest that elevation in the CPD-dependent oxidative phosphorylation and fatty acid oxidation is due to the activation of CPD-dependent pathways regulating carbohydrate and lipid catabolism.

In addition to increased carbohydrate and fatty acid metabolism, we investigated the role of lipid droplets, which are one of the most important energy stores of cells during nutrient deprivation. Confocal microscopy revealed a CPD-dependent increase in the number and size of lipid droplets. In parallel with lipid droplet biogenesis, we detected a similar change in the number and size of autophagosomes. We observed colocalization between lipid droplets and autophagosomes referring to a special form of autophagy, called lipophagy.

Accumulation of lipid droplets is observed during keratinocyte differentiation. Immunofluorescence analysis confirmed that the expression of early differentiation marker keratin 1 (K1) is simultaneously upregulated with cellular lipid droplet content. By investigating the relationship of LDs and keratinocyte differentiation on mitochondrial activity, we found that Olaparib, a PARP inhibitor, induced K1 expression and lipid accumulation, in agreement with its capacity to induce hypermetabolism. Oxaloacetate and acetyl-CoA, which are anaplerotic substrates and members of the citrate cycle, similarly induced K1 expression and lipid accumulation. Finally, pharmacological inhibition of the CPD-modulated energy-sensor enzymes impacted on K1 expression and lipid accumulation. Any compounds that inhibit mitochondrial catabolism reduced the amount of lipid droplets and K1 protein as well, suggesting that keratinocyte differentiation and lipid droplet accumulation are tightly interconnected and regulated in a CPD and mitochondrial activity-dependent manner.

Finally, we detected increased CPD-dependent reactive oxygen species (ROS) production in UVB-treated cells. Increased ROS production was abolished when cells were treated with strong reductants, such as reduced glutathione (GSH), N-acetyl-cysteine (NAC) or a mitochondrial reactive species scavenger, MitoTEMPO, suggesting that reactive species production partially originated from the mitochondria. Other hallmarks of UVB-induced, CPD-dependent hypermetabolic switches, such as MitoTracker Green fluorescence (marker for mitochondrial biogenesis, mitochondrial content), MitoTracker Red CMXRos fluorescence (a readout of mitochondrial membrane potential), induction of ECAR (glycolysis), and basal OCR (oxidative phoshorylation) were abrogated upon GSH, NAC, and MitoTempo treatment. Similarly, lipid deposition and increased LC3 expression (autophagy) were also prevented by GSH, NAC, and MitoTempo treatment. Taken together, these data demonstrate that ROS, originating partially from the mitochondria, play a role in CPD-dependent compensatory reactions to UVB treatment.

In our work, **we demonstrated the relationship between UVB-induced acute DNA damage and mitochondrial changes.** CPD removal by photolyase activation inhibited UVB-induced mitochondrial biogenesis, fusion, and hypermetabolism. We identified the role of multiple pathway in the regulation of autophagy, lipid droplet biogenesis, keratinocyte differentiation, and mitochondrial activity. These results suggest that, introduction of the photolyase enzyme into the skin, using antioxidants, and the inhibition of certain metabolic pathways may have therapeutic potential in the UVB response(77).

3. Plant extract and small molecules can inhibit nucleotide excision repair (NER) but their cellular biological effects can be unpredictable from their repair inhibitory effects.

3.1. There were no UVB-damage-mitigating effects of a phyto-polyphenol compounds in vitro on human keratinocytes when used before or after UV irradiation. Instead of protective effect of silymarin, we identified its strong phototoxicity under UVA irradiation.

We hypothesized that our collaborator's phyto-polyphenol extract with known ROS scavenging activity has protective effect not only when applied before, but also when applied after the UVB irradiation and increases post-UVB survival.

Our collaborators from the Department of Pharmaceutical Technology have already extracted phyto-polyphenol compound (*Silybum marianum* plant extract) and carried out preliminary experiments proving the UVB protective effects of the active ingredient measured on an animal model detecting decreased ROS production(78). Interestingly, better protection was seen when the active ingredient was present both before and after UVB effect. This finding is unique among most known antioxidants. Silymarin also recognized as UVB protective compound by scientific literature. We wanted to study whether the active ingredient of the phyto-polyphenol compound developed by pharmacist (79) can decrease UVB induced changes, whether the active ingredient modifies CPD formation and elimination when administered after UVB

irradiation. In order to address this question first we performed basic viability experiments with UVB and UVA irradiation using these compounds.

The *Silybum marianum* plant extract induced phototoxicity on UVA-exposed keratinocytes. Silymarin, originating from milk thistle, contains several bioactive components, such as silibinin, silychristin, silydianine and taxifolin. The antioxidant, anti-inflammatory, anti-fibrotic and immunomodulatory effects of the complex is widely-known. However, the poor solubility of silymarin in water, thus its low bioavailability makes the dermatological application of the compound difficult ((80). Dermatological application of silymarin is also controversial: antioxidant, photoprotective (81) and phototoxic (82) effects of the compound were also shown. After experience of phototoxicity of silymarin our further aim was to evaluate the effects of silymarin compounds with different origins on the viability of UVA and UVB-irradiated human keratinocytes. For measurements, four different topical formulations of silymarin containing penetration enhancers were prepared by the Department of Pharmaceutical Technology (University of Debrecen).

We examined the effects of silymarin extracts from three different origins: 1) silymarin powder ordered from Sigma-Aldrich, 2) commercially available, silymarin-containing dietary supplement (Silegon) and 3) four topical formulations of silymarin containing penetration enhancers (Department of Pharmaceutical Technology) on UVB- or UVA-irradiated HaCaT keratinocytes. Silymarin treatment did not affect UVB-induced cell death, but it induced significant, dose-dependent decrease in cell viability after UVA treatment. Silymarin (Sigma) and Silegon had similar effect on cell survival. In the case of silymarin dissolved in penetration enhancers, the extent of phototoxicity varied based on the composition of the different formulations. It suggests that appropriate formulation contributes to the bioavailability of the phyto-phenol.

Silymarin had antioxidant capacity. We evaluated the effects of silymarin compounds on UVA-induced ROS (reactive oxygen species) production. Silymarin pre-treatment significantly decreased the amount of intracellular ROS after high-dose (20 J/cm^2) of UVA. Decrease in ROS was observed in the case of all silymarin compounds.

Silymarin enhanced CPD accumulation, but had no effect on the number of mutation. To assess the effect of silymarin on UVA-induced DNA damage, relative amount of UVA-induced CPD lesions was quantified 24 hours after 10 J/cm^2 UVA. Silymarin treatments significantly enhanced CPD accumulation after irradiation, which suggests that the phyto-phenol compounds may also inhibit NER function. Effects of the extracts on UVA-induced mutational burden was also assessed by HPRT gene mutation assay. Despite the previously observed, enhanced CPD accumulation, silymarin had no effect on the number of mutation-carrying cells after UVA.

Effect of silymarin on cellular UV-response is controversial: the antioxidant and photosensitizing abilities of silymarin were also shown. The reason of increased CPD accumulation by silymarin treatment after UVA radiation is currently unknown. However, neutral effect of silymarin on UVA-induced mutagenesis suggests that elevated number of CPDs does not necessarily leads to enhanced risk of skin cancer. Aldought *Silybum marianum* plant extract had antioxidant effect on UVA exposed keratinocytes, it also induced profound phototoxicity, but did not increase UV induced mutation.
According to our results, dermatological applications of natural flavonoids require careful testing and thoughtful assessment of their potential UV interaction to avoid possible adverse effects(83).

3.2. Small molecular inhibitors of NER instead of increasing UVB irradiated cellular DNA damage, unexpectedly do not increase UVB induced mutation but opposite it even some of them reduce in vitro mutation burden of cells. Their effect on cell cycle delay, apoptosis, and autophagy may partly explain this finings.

The long-term effect of CPD lesions greatly depends on the function of intracellular repair mechanisms. Chemical inhibition, thus decreased activity of the repair proteins may result in enhanced UV-induced mutagenesis. PARP1 inhibitors(84), resveratrol(85), spironolactone(86) and inorganic arsenic compounds (such as arsenic-trioxide)(87) are clinically used molecules, which are proved to inhibit the nucleotide excision repair (NER) complex. Our aim was to demonstrate the NER inhibitor capacity of veliparib, resveratrol, spironolactone and arsenic-trioxide; and investigate their effects on UVB-induced mutagenesis of CHO (Chinese hamster ovary) and HaCaT (immortalized human keratinocyte) epithelial cells *in vitro*. We also aimed to investigate the effects of these compounds on UVB-induced apoptosis, cell cycle arrest, autophagy and protein expression changes.
In our work, HaCaT and CHO cells were pre-treated with the inhibitor molecules for 2 hours prior to 10-20 mJ/cm^2 UVB irradiation, depending on the experiment. After veliparib, resveratrol, arsenic-trioxide or spironolactone treatments, the relative amounts of CPDs were significantly higher compared to the control (UV-irradiated only) cells, suggesting the inhibitory effect of the treatments on CPD-repair. To assess UVB-induced mutagenesis, previously established (1st and 2nd years of the project) HPRT gene mutagenesis assay was performed(88). We observed that the mutagenic effect of UVB and CPD accumulation did not exhibit parallel increase, as UVB-induced mutagenesis decreased at higher UVB doses. We also found that three out of four inhibitor molecules (veliparib, arsenic-trioxide, spironolactone) significantly decreased UVB-induced mutagenesis. Higher concentrations of the compounds decreased the mutation rates almost to baseline. Resveratrol did not cause any changes in UVB-induced mutation formation. Besides, arsenic-trioxide and spironolactone treatments decreased cell viability with 40%, compared to control (UVB-irradiated only) cells. Resveratrol induced mild decrease in cell viability. By cell cycle measurements, veliparib caused a significant increase in the percentage of cells in the G2/M phase, suggesting that the treatment augments UVB-induced cell cycle arrest. The increase lasted up to 6 days after the exposure. Resveratrol caused a moderate elevation in G2/M-block 3 days after the UVB.

In our further work, we aimed to identify general upstream regulators of UV response at the protein level. We measured total protein expression and phosphorylation status of p53, LC3III („microtubule-associated protein 1A/1B-light chain 3 protein") and mTOR („mammalian target of rapamycin"). Alterations were assessed 2, 6 and 20 hours after 20 mJ/cm^2 UVB irradiation. Veliparib pre-treatment caused significant changes in p53 phosphorylation level. Other treatments did not affect p53 activation. LC3-II autophagy marker was also significantly increased after veliparib treatment. Arsenic-trioxide and spironolactone enhanced mTOR phosphorylation after UVB irradiation, but statistically significant difference was only detected in the case of spironolactone treatment.

These results show that there is no obligate linear relationship between repair activity and UVB mutagenesis, but other factors should be also considered while assessing the possible risk of skin cancer induction by a chemical treatment. We published our work in a peer-reviewed, open access scientific journal and also presented in hungarian dermatologic conferences as an oral peresentation(73).

4. Ablative fractionated Er:YAG laser improve the effect of photodynamic therapy (PDT) clinically and histopathologically. Skin immune infiltration was characterized in PDT treated precancerous skin. Langerhans cells and CD3$^+$ T cells in AKs was seemed to associate with higher PDT efficacy

High cumulative lifetime sun exposure increases the risk of precancerous actinic keratosis (AK) and the definitive cancer, squamous cell carcinoma (SCC) too(89). Certain circumstances, like immunosuppression also contribute to the development of skin tumors(90). Although, the mortality of non-pigmented skin cancers is low, their treatment has a high economic burden on the society(89). Therefore, we have to identify preventive measures to decrease the appearance of actinic keratosis and skin tumors.
There are multiple AKs on severely sun damaged skin, which reflect to field carcinogenesis, that means that not only AKs but the surrounding normal looking skin should be treated as well, because it's

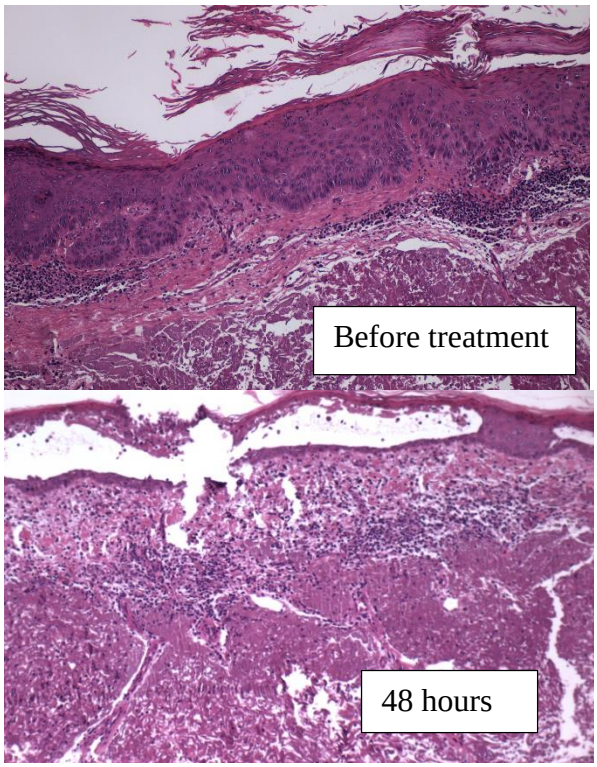
keratinocytes may contain certain amount of DNA damage too(91). Therefore field treatment, like photodynamic therapy (PDT) is needed to effectively treat this area(92).) Thus, PDT can also be considered as a tool for primary prevention. A few scientific evidences showed Er:YAG (erbium:yttrium-aluminium-garnet) ablative fractional laser - assisted PDT increases the therapeutic efficacy of conventional PDT (93). This was attributed to better photosensitizer delivery and conversion.
We have special clinics for organ transplant recipients (OTR) and patients with multiplex skin tumors and precancers (field cancerization) and collected clinical data for scientific use(94) and performe field treatments.

First, we studied the differences between conventional PDT and Er:YAG ablative fractional laser - assisted PDT both in efficacy and rejuvenation effect. Sixteen patients with multiple AK and severely sun-damaged skin were included in the study. 20% 5-ALA was applied on half side of the scalp, face, dorsum of the forearms and hands and then was irradiated with water-filtered infrared A (wIRA) light (Hydrosun® 501 halogen lamp for 20 minutes after 4 hour incubation time, while the other side was pre-treated with Er:YAG-AFL (ablation depth: 30-100 μm; density: 22%) before PDT. We assessed the number and grade of AK and the objective signs of photoaging before, 3 and 12 months after therapy. Skin biopsy samples were obtained at 24/48 hours and 3 months later. There was no statistically significant difference in the initial number of AK. The number of AK has significantly decreased 3 and 12 months after the laser-assisted PDT and also after conventional PDT. The efficacy of Er:YAG-AFL PDT to reduce the number of AK was significantly greater than conventional PDT at 3 months control but not at 12 months follow-up. Both treatment modality significantly improved global photoaging (94).
In a review, we summarized the major effects of PDT on keratinocytes, innate and adaptive immune cells(95). Analyzing the literature, human data were not available neither in the context of immunological effects of PDT on DCs in AK or cSCC, nor the composition of T cells in AKs or the influence of PDT on T cells in human AK samples were available. We found that major events induced by PDT were extensively studied in non-skin cancer models both in humans and animals, while the immunological effects of 5-ALA/MAL PDT on AK, intraepithelial cancer and cutaneous SCC lesions have been mainly investigated in mouse models. Experimental data in human were missing.
Therefore, in our further investigations, we extended the histopathological evaluation on immune infiltrate besides clinical evaluation. The main objective of the study was to analyze and compare the composition of immune cells infiltrating AKs before and after conventional and Er:YAG -AFL - assisted PDT in order to assess, how PDT with these modalities could change the quality and quantity of induced immune response. We also aimed to compare therapeutic efficacy and photorejuvenation effect of Er:YAG - AFL compared to conventional PDT (cPDT) in patients with multiple actinic keratosis during a 12-month follow up period. Treatment area were randomized to receive conventional PDT (cPDT) or Er:YAG-AFL PDT in random manner. Patients were evaluated 1, 3, 6, 9 and 12 months after treatment by the same investigator. The number and grade of AKs were determined on all visits on both treated sites without knowing the form of treatment. The objective signs of photoaging were evaluated before, 3 and 12 months after therapy. Five-point scale for photodamage were applied to determine the objective signs of photoaging. Skin biopsy samples (6mm punch biopsies) of an AK and photographically verified sites of previous AKs were obtained before PDT, 48 hours and 3 months after PDT. Eleven patients (average age $77\pm6{,}9$ years) with a total number of 427 AKs completed the study. There was no statistically significant difference in the initial number of AKs on the two differently treated sides. The number of AKs has significantly decreased 3 and 12 months after PDT both sides Er:YAG AFL pretreatment induced higher therapeutic efficacy in those patients where PDT was highly effective.
Both treatment modalities significantly improved photoaging 3 months later. Analyzing hematoxylin-eosin-stained tissue sections we found prominent dysplasia and solar elastosis, and a moderate inflammatory cell infiltration in AK samples before treatment. 48 hours after Er:YAG-AFL PDT or cPDT, a prominent inflammation with acantholysis and necrosis could be observed. 3 months later, both dysplasia and solar elastosis decreased. The number of p53 positive keratinocytes significantly decreased at 48 hours and it was also significantly lower at 3 months after both compared to the corresponding initial AK field. The number of Ki67 positive cells decreased significantly at 48 hours and was significantly lower at 3 months after Er:YAG-AFL-PDT compared to the pre-PDT AKs. The ratio of Ki67, CD1a, CD3,CD4, CD8

immunopositivity was analyzed. Fig. 1. graphically summarized the resulst and Fig 2 shows photos of representative hematoxillin esoin stained slides.
Although the number of $p53^+$ and $Ki67^+$ epidermal cells has substantially decreased by PDT showing the decreased number of damaged cells less most pronounced in Er:YAG-AFL PDT group compared to the initial status, but the treatment did not eliminate the abnormal cells totally, suggesting one treatment session is not enough to eliminate actinic damage, and therapy should be repeated. This observation is accordance with clinical experiences. Photodynamic therapy induced immunological changes. It seems that the increased number of $CD1a^+$ Langerhans cells and $CD3^+$ T cells in AKs is associated with higher PDT efficacy(96).

Cells	48 hours	3 months
$CD1a^+$	↓↓	↑↑
$CD3^+$	↓	↓
$CD4^+$	↓	↓
$CD8^+$	↓	↓↓

Figure 1. PDT induced changes in immune infiltrate



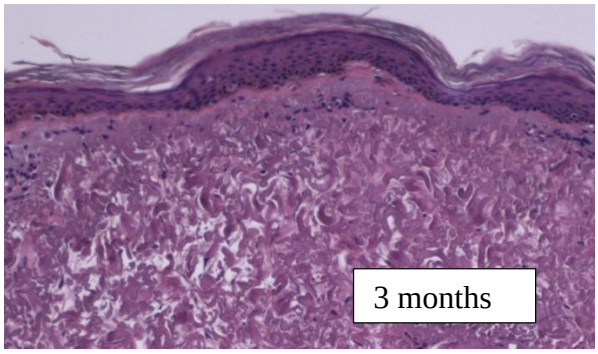


Figure 2. Effect of Er:YAG-AFL-PDT on AK 48 hours and 3 months later (H&E staining, 100x)

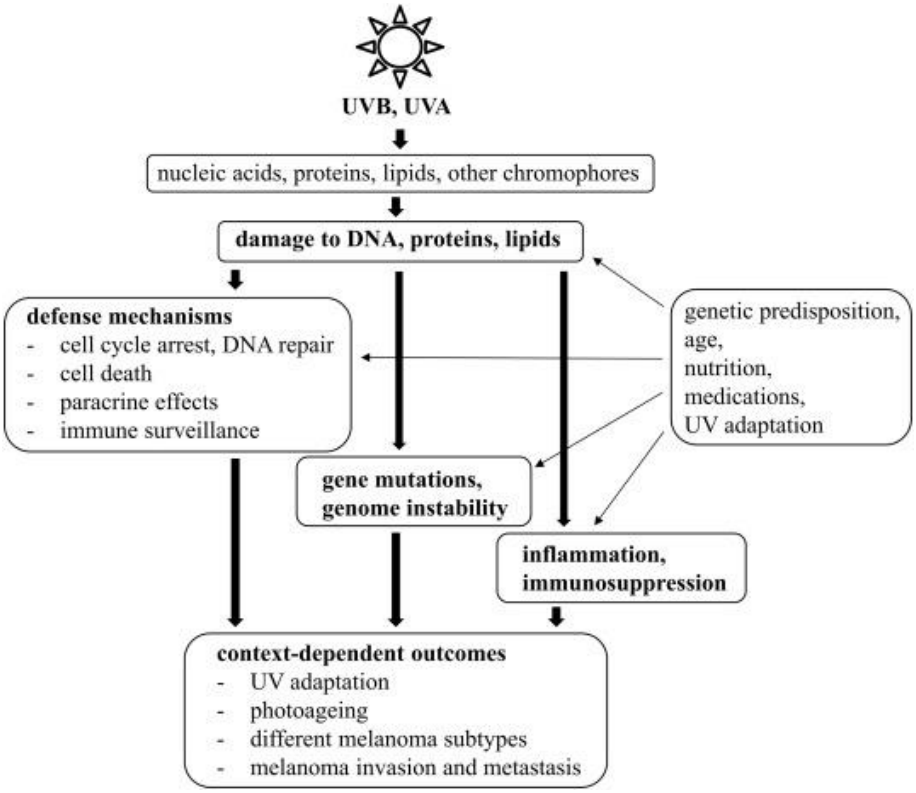
5. Epidemiology studies in melanoma malignum.

5.1. Screening of a high-risk group is more cost-effective, based on our results it would be desirable to screen melanomas among those over 65 years of age.

Ultraviolet light is an important factor in the development of cutaneous malignant melanoma (CMM) of which the incidence has been greatly increasing worldwide. Most UVB radiation is absorbed by the epidermis, 9–15% of UVB and approximately 50% of the UVA radiation of sunlight reaches melanocytes in the basal layer. The carcinogenic effects and the biological effects of UV light are not only determined by the physical properties of the UV exposure, numerous other factors including genetic predisposition, age, nutrition and medications modify the effects of UVR. Both UV-induced gene mutations in melanocytes and changes caused in the immune microenvironment may play a role in the tumor development. The degree of UV-induced stress and the protection against stress are influenced both by intracellular and intercellular molecular interactions.

Nevertheless, cutaneous melanoma is not a homogenous disease, and the interaction of variable environmental exposure and different genetic susceptibility and other host factors lead to the formation of melanomas with different biological behavior and clinical characteristics.

Although the targeted and immune checkpoint therapies revolutionized metastatic melanoma treatment several patients fail to respond to these modalities and still primary and secondary prevention is extremely important in saving lives of the patients. Epidemiological data help to determine effective prevention strategies(97).



Source: Emri et al.: Ultraviolet radiation-mediated development of cutaneous melanoma: An update. J Photochem Photobiol B. 2018 Aug; 185:169-175(97).

Metallothioneins (MTs) belong to a group of small cysteine-rich proteins that are ubiquitous throughout all kingdoms. The main function of MTs is scavenging of free radicals and detoxification and homeostating of heavy metals. In our previous work we have shown that overexpression of MT is significantly increased in primary CMM with haematogenous metastases and that the overexpression is independent of the Breslow tumor thickness. MT overexpression of the tumor cells was correlated with the presence of tumor-infiltrating CD68(+) macrophages. A comprehensive summary of methods for analysis of MTs at (sub)isoforms, levels, their expression in single tumor diseases and strategies how this knowledge can be utilized in anticancer therapy give better understanding of the mechanisms and consequences of UV-induced DNA-damage and may contribute to improve melanoma primary prevention and therapy(98).

Cutaneous melanoma is a cancer arising from the malignant transformation of skin melanocytes. Historically, melanoma was a rare cancer. However, in the last 50 years, the incidence of melanoma has risen faster than most other cancer. Although melanoma still represents less than 5% of all cutaneous malignancies, melanoma accounts for the majority of skin cancer deaths. Changes in the incidence and prevalence of melanoma are not uniform across the world, even across Caucasian populations and similar geographical locations. The inconstancies in melanoma prevalence and incidence have not been fully explored.

We assessed the incidence, type and localization of diagnosed melanoma at the Department of Dermatology University of Debrecen, in the period 2000-2014. Using the melanoma register of the Department of Dermatology Faculty of Medicine University of Debrecen, we studied retrospectively the trends in the incidence and characteristics of patients (n=1464) and their melanoma in North-East Hungary from 2000 to 2014. The incidence, Breslow tumour thickness, stage of disease at initial diagnosis (based on American Joint Committee on Cancer 7th edition TNM staging classification), age distribution, gender, localization and type of melanomas were analyzed and trends were calculated by dividing the investigated period into 5 parts, in which every part consist of 3 years. Standardized incidence rates were calculated using the data of the NCR, the Central Statistical Office of Hungary and the Standard World population. We found significant increase in melanoma incidence in our region with a breakpoint in 2007. From 2001 to 2007 the trend was increasing (APC 9.84 [3.52; 16.55]; p=0.006), which stalled from 2007 (APC: -2.45 [-5.99; 1.23]; p=0.164). Our results showed a favorable tendency for tumor thickness in the second half of the 2000s, but this trend reversed during the 2012-2014 period. The rate of new melanoma cases was significantly higher

among older people. Our results confirm the need to strengthen educational and screening programs in Hungary. Since cancer screening of a high-risk group is more cost-effective, based on our results it would be desirable to screen melanomas among those over 65 years of age(99).

5.2. It was confirmed that melanoma prevalence can be influenced by socioeconomic status.

In the case of Hajdú-Bihar county, our research group has already performed the analyzes for a previous study period, and during the preparation of the publication the question arose whether the characteristics and changes experienced in our study region can be found. in counties with different economic and geographical conditions than our region.
We carried on the epidemiological and biomarker studies on malignant melanoma. Anonymized patient records from the National Health Insurance Fund Management covering the entire population were used to determine the incidence and prevalence of melanoma in the counties of Hungary from 2013 to 2017. Altogether 20,030 melanoma cases were identified for inclusion in this study. The prevalence of melanoma increased over the investigated period and was significantly higher among women than men. The incidence of melanoma stagnated during this period and the incidence rate was the highest among the elderly. Interestingly, the incidence was higher in males in the elderly population, while the incidence was higher in females in the younger (<60 years) population.
Geographical variations in ambient UV radiation did not show statistically significant correlation with the regional variability of epidemiological indicators, probably due to small differences in the number of bright sunshine hours per year between regions. Although Hungary is a relatively small country, we observed regional heterogeneity in socioeconomic factors. Notably, a significant and strong negative correlation was found between single-person household rates and melanoma prevalence. In addition to ambient UV radiation, melanoma incidence and prevalence appear to be related to age, gender, and socioeconomic factors.
In conclusion, melanoma is a multifactorial disease; thus, the pathogenesis of melanoma is also heterogeneous. The three main influencing factors are environmental (UVR), genetic, and socio-demographic factors. UV exposure is undoubtedly strongly associated with melanoma development, but the trend in the incidence of melanoma does not simply follow the level of ambient UVR, highlighting the effects of individual risk factors. We found regional differences in our relatively small country, so it is not surprising that melanoma epidemiological trends varying worldwide. Importantly, our data confirm that melanoma prevalence can be influenced by socioeconomic status, suggesting that there are biological factors in the pathogenesis of melanoma that can be affected by socioeconomic environment.
In the European Journal of Cancer Prevention was accepted the manuscript(100).

5.3 Serum S100B had a similar and independent prognostic strength in metastatic melanoma compared with serum LDH.

Furthermore, a systematic review and meta-analysis were performed. A quantitative analysis of data from 6 eligible studies included 1033 patients with cutaneous melanoma. The discriminative ability of serum S100B at identifying disease relapse was acceptable (pooled Area Under the ROC (AUROC) 78.64 [95% CI 70.28; 87.01]) and significantly higher than the discriminative ability of serum LDH (AUROC 64.41 [95% CI 56.05; 7278]) (p=0.013). The pooled sensitivity of serum S100B was significantly higher than the sensitivity of LDH (p=0.017). Ten eligible studies with 1987 patients were included in the risk of death analysis. The prognostic performance of serum S100B (pooled estimate of adjusted hazard ratio (HR) 1.78 [95% CI 1.38; 2.29]) was independent but not superior to that of serum LDH (HR 1.60 [95% CI 1.36; 2.29]). Serum S100B is a useful biomarker in melanoma patients. Serum biomarkers may provide relevant information on melanoma patient status and should be further researched.
The novelty of this meta-analysis was the comparative approach, the analysis of multiple outcomes, and the inclusion of logistic regression models. Furthermore, the results were derived from the analysis of data from patient populations with more than 1000 participants for each of the studied outcomes.

The applicability of serum S100B and serum LDH for predicting the progression of melanoma was studied in this review from both diagnostic and prognostic viewpoints. We found that the discriminative ability of serum S100B at identifying disease relapse was greater than that of serum LDH. Since a relapse of melanoma is associated with elevated serum S100B levels in only a subset of patients, serum S100B should be considered in combination with additional serum biomarkers in a multivariable diagnostic prediction model. Furthermore, serum S100B had a similar and independent prognostic strength in metastatic melanoma compared with serum LDH, suggesting that the implementation of both markers in a multivariable prognostic prediction model development would be advantageous. To increase the degree of confidence in the prognostic and diagnostic abilities of various biomarkers, primary predictor studies conducted and reported in accordance with the corresponding quality assessment tools are important.
These results hopefully will be published in Frontiers in Oncology, the manuscript (101) has already been submitted to the journal.

APPLIED METHODS

In vitro synthetized pseudouridine modified CPD photolyase **mRNA transfection** was carried out by lipofectamin based method on human keratinocytes (HaCaT, HNEK) and other epithelial cell (CHO) according to previous publication(66, 76) with slight modification and adaptation to recent study requirements . Broad band UVB and UVA irradiation on the cell cultures. We used **ELISA** technique, and **flow cytometry** to detect the amount of CPDs in cellular DNA and cell viability and also **apoptosis assays** were performed. At an intermediate time point after CPD removal we have done qRT- PCR to analyze gene expression, **and Western blot** or **immunocyto- or histochemistry to detect protein expression**. **Mitochondrial membrane potential and reactive oxygen species** were determined. Electron and confocal microscopy were used for characterisation of the mitochondrial ultrastructure, fusion and fission. XF96 oximeter for measuring OCR/ECAR, colorimetric and fluorometric assays for detecting NAD, ATP level. HPRT gene mutation assay was established to detect UVB driven mutation in cultured cell line.
For the epidemiology studies our melanoma register was used supplemented with the hospital information system used in the University of Debrecen. Furthermore, for the standardization we used more international and Hungarian database, such as the Standard World population, the Central Statistical Office of Hungary (number and age distribution of inhabitants), National Cancer Registry and National Health Insurance Fund Management (incident cases). Standardised incidence rates in Hungary were calculated per 100,000 people and age-adjusted to the world standard population by direct standardisation. For counties, the incidence rate of melanoma standardised by age and gender (per 100,000 people) was calculated using indirect standardisation compared to the Hungarian data. Trends were analysed by joinpoint regression, which determines the average annual percentage change (AAPC) and the corresponding 95% confidence intervals. The statistical analysis were performed using SPSS 25 (SPSS program, Chicago, IL, USA), GraphPad Prism 7.0 (GraphPad SoftwareVersion 7.0, San Diego, USA), EpiInfo (Epi Info™, Division of Health Informatics & Surveillance (DHIS), Center for Surveillance, Epidemiology & Laboratory Services (CSELS)). AAPCs were calculated using the Joinpoint Regression Program (Version 4.4.0.0 Statistical Methodology and Applications Branch, Surveillance Research Program, National Cancer Institute; January 2017, USA).

RESEARCH INFRASTRUCTURE

All the included methods and models are available and have been previously established.
Department of Dermatology, University of Debrecen:
Histopathology Laboratory: cryostat, microtome, automated paraffin embedded system, microscopes, immunofluorescence, immunoperoxidase, image documentation software
UV irradiation: Light sources: UVB lamps (TL20W/12 RS broadband Philips, Germany), DayLight Fluorescent lamps (Sylvania, F18W); radiometer (UVP Inc. San Gabriel, USA)
Cell culture facility: CO2 incubator and laminar flow, incubators
Molecular biology and chemistry laboratory: Gel Documentation system, ELISA reader (Anthos 2020), Western blot, Nanodrop, PCR instrument; Flow cytometer (Cell Therapy Unit, in the building of the Dept. of Dermatology)

Collaborator partner's laboratory:
Department of Pharmaceutical Technology, University of Debrecen
FLUOstar OPTIMA Microplate Reader for the xCELLigence system (Roche, Switzerland), E-plates (Roche, Switzerland) Franz diffusion cell system Brookfield texture analyzer
Core facilities of the University of Debrecen:
Mitochondrial function: XF96 oximeter, fluorimeters, cytometers for superoxide production and mitochondrial charge determination **(**Dept. of Medical Chemistry),ABI 7900HT real time quantitative PCR instruments (Dept. of Biochemistry and Molecular Biology, Genomics Center),RNAseq, DNA sequencing (Dept. of Biochemistry and Molecular Biology; Genomics Center), Laser scanning cytometer (Dept. of Biophysics and Cell Biology)
Confocal Microscope (Inst. of Biophysics)

SUMMARY

We identified molecular and cellular mechanisms capable of reducing the consequences of UVB irradiation *in vitro*. UVB causes DNA damage (cyclobutane pyrimidine dimers (CPD)), decreases viability of keratinocytes and also induces mutations. *In vitro* synthesized pseudouridine-containing CPD specific photolyase mRNA transfection, developed by us uniquely, effectively removes CPDs within 6 hours after UVB irradiation. The multifaceted role of poly-(ADP)- ribose polymerase-1 in UVB response were demonstrated. We also proved that UVB driven metabolic changes depend on CPDs and mitochondria as the secondary source of UVB-induced ROS. Plant extracts and small molecules can inhibit nucleotide excision repair but their cellular biological effects can be unpredictable from their repair inhibitory capacity. Some of them not induce, even protect against mutations. *In vitro* photosensitivity of silymarin extracts warn the scientific dermatological community about careful usage them on human skin. Clinical and histopathological evidences were provided for improved effectiveness of ablative fractionated laser assisted photodynamic therapy (PDT) and were characterized the immune infiltration after treatment. Epidemiological studies on melanoma suggested to screen melanomas among those over 65 years of age and that melanoma prevalence can be influenced by socioeconomic status. Serum S100B had a similar and independent prognostic strength in metastatic melanoma compared with serum LDH.

INDIRECT ECONOMIC BENEFITS:

There is currently no direct economic exploitation of our results, but if transepidermal nucleic acid delivery is achieved, sunscreen can be developed. Therapeutic application of mRNAs has already patented by Katalin Kariko and Drew Weismann in 2012..
Using a fractionated laser, it is likely to be necessary to repeat the PDT less frequently.
There is a public expectation for melanoma screening, with our data supporting that population wide screening is not worth introducing, but specific attention need for those over 65, it is worth drawing the attention of GPs and dermatologists to the importance of dermatological examination in this ages.

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