Ashish Dwivedi · Neeraj Agarwal
Lipika Ray · Amit Kumar Tripathi *Editors*

Skin Aging & Cancer

Ambient UV-R Exposure

Springer

Skin Aging & Cancer

Ashish Dwivedi • Neeraj Agarwal
Lipika Ray • Amit Kumar Tripathi
Editors

Skin Aging & Cancer

Ambient UV-R Exposure

Springer

Editors
Ashish Dwivedi
Department of Zoology
Banaras Hindu University
Varanasi, Uttar Pradesh, India

Lipika Ray
Pharmaceutics and Pharmacokinetics Division
CSIR-Central Drug Research Institute
Lucknow, Uttar Pradesh, India

Neeraj Agarwal
Department of Medicine, Division of Hematology/Oncology
Samuel Oschin Comprehensive Cancer Institute Cedars-Sinai Medical Center
Los Angeles, CA, USA

Amit Kumar Tripathi
School of Biomedical Engineering
Indian Institute of Technology BHU
Varanasi, Uttar Pradesh, India

ISBN 978-981-13-2540-3 ISBN 978-981-13-2541-0 (eBook)
https://doi.org/10.1007/978-981-13-2541-0

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

It gives us immense pleasure to write the first edition of the book entitled *Skin Aging & Cancer: Ambient UV-R Exposure.*

Skin is the largest human organ which offers protection to the body. However, our skin naturally ages with time which promotes skin inflammation, impaired wound repair, and increased risk of skin cancer. Skin aging occurs by multi-factorial processes, involving environmental and genetic factors. One of the major environmental factors causing skin aging is ultraviolet radiation (UV-R) coming through sunlight which damages sun-exposed skin areas and promotes photo-induced aging or photoaging. Chronic UV-R exposures on skin often lead to skin cancer. Incidences of skin cancer are increasing globally, and major causing factors are increased outdoor activities, ozone depletion, genetic alterations, and immune suppression. This book will educate about how UV-R initiates cancer, mechanisms involved, potential therapy, and what protective measures can be taken. In this book, we discuss the role of UV-R-induced skin aging and cancer to provide insights into the pathogenesis and process to reduce photoaging. Furthermore, this book also describes nanotechnology-based therapeutic development of skin cancer in the recent era and bioinformatics explanations of skin aging and cancer.

All the authors in this book are highly skilled researchers with thorough and up-to-date knowledge in the field and are actively doing research in the relevant fields. For all the chapters, we put our best efforts to provide collated information from research articles published so far related to the subject and arranged them in subsections to make them easy to follow. We have tried our best to use the simplified language so that it can be understandable to all levels of readers.

We hope that after reading the book, the reader will have more knowledge, understanding, and awareness about the harmful effects of repetitive or chronic UV-R exposure as well as its interactions with other chemicals and drugs on the skin or inside the body.

Varanasi, Uttar Pradesh, India — Dr. Ashish Dwivedi
Los Angeles, CA, USA — Dr. Neeraj Agarwal
Lucknow, Uttar Pradesh, India — Dr. Lipika Ray
Varanasi, Uttar Pradesh, India — Dr. Amit Kumar Tripathi

Acknowledgments

The editors are heartily thankful to all the authors and reviewers for investing their valuable time and hard work in writing and reviewing the chapters for this book. Without their support, this book would not have become a reality. I highly admire that all authors took extra stress to provide detailed information based on their research knowledge and literature from recent publications to make all the chapters in this book up-to-date and more valuable for its readers.

Contents

About the Editors

Dr. Ashish Dwivedi is a Postdoctoral Scientist in Skaggs School of Pharmacy and Pharmaceutical Sciences, Colorado University, Anschutz Medical Campus, Aurora, CO, USA. He has done his Doctoral Research from Photobiology Division, Indian Institute of Toxicology Research (IITR), Lucknow. His doctoral research work was focused on phototoxicity assessment of different therapeutic drugs and environmental pollutants. He has 11 years of research experience in photosciences and published many papers in reputed journals of *Photochemistry and Photobiology*, *Toxicology*, *Biomaterials*, *Hazardous Materials*, etc.

Dr. Neeraj Agarwal is working as a Scientist at NCI-designated University of Colorado Comprehensive Cancer Center, Aurora, CO, USA. He completed his Ph.D. (2001–2007) from Photobiology Division of IITR, Lucknow. His work was focused on determining the phototoxic potential of commonly used therapeutic drugs and their mechanism of action. He has published seven research papers and presented his work at various conferences. He received the best poster award in 2002 for the work related to ciprofloxacin phototoxicity. In 2007, he joined Louisiana Health Sciences Center (LSUHSC), New Orleans, LA, USA, as a Postdoctoral Researcher and worked for 5 years towards understating the role of MTBP protein in osteosarcoma metastasis and also on identifying and characterizing the cancer stem cells in osteosarcoma. He received the Scientific Excellence Award and was invited to present his work in Cancer Center Retreat in 2010. Till now, he has published 18 research articles in reputed international journals with high impact factors, including *Cancer Research*, *Oncogene*, *Cell Death and Differentiation*, *Clinical Cancer Research*, and *Photochemistry and Photobiology*.

Dr. Lipika Ray is a Scientist in Pharmaceutics and Pharmacokinetics Division, CSIR-Central Drug Research Institute (CSIR-CDRI), Lucknow. She has expertise in the field of nanomedicine. She has done post-doctoral research as a DST-Young Scientist in Photobiology Division, IITR, Lucknow. The project was mainly focused on phototoxicity assessment of bulk and nano-curcumin. She has 15 years of research experience and published many papers in peer-reviewed journals such as *Biomaterials, International Journal of Pharmaceutics*, *European Journal of Pharmaceutics*, and *Biopharmaceutics*.

Dr. Amit Kumar Tripathi is a Research Scientist in the Department of Internal Medicine, Carver College of Medicine, The University of Iowa, Iowa City, IA, USA. He has done his Doctoral Research from Cell Death Research Laboratory, Endocrinology Division, CSIR-Central Drug Research Institute, Lucknow, India. His doctoral research was focused on the mechanistic studies to understand the neuroprotection. He has 10 years of research experience in rodent models of various neurological disorders such as transient middle cerebral artery occlusion, cerebral venous sinuses thrombotic, traumatic brain injury, and Parkinson disease. He has published his research work in various peer-reviewed international journals, including *Journal of Biomedical Science* and *Neuroscience Letter*.

Contributors

Neeraj Agarwal Department of Medicine, Division of Hematology/Oncology, Samuel Oschin Comprehensive Cancer Institute Cedars-Sinai Medical Center, Los Angeles, CA, USA

Saroj Kumar Amar Department of Forensics Science, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

Monisha Banerjee Molecular and Human Genetics Laboratory, Department of Zoology, University of Lucknow, Lucknow, India

Shilpa Chakravarty Biochemistry Department, University of Allahabad, Prayagraj, India

Deepti Chopra Photobiology Laboratory, Systems Toxicology and Health Risk Assessment Group, CSIR-Indian Institute of Toxicology Research, Lucknow, India Babu Banarasi Das University, Lucknow, India

Divya Dubey Photobiology Laboratory, Systems Toxicology and Health Risk Assessment Group, CSIR-Indian Institute of Toxicology Research, Lucknow, India Babu Banarasi Das University, Lucknow, India

Kailash Chand Gupta CSIR-Institute of Genomics and Integrative Biology, New Delhi, India

Dhanananajay Kumar Department of Pharmaceutical Engineering & Technology, IIT-BHU, Varanasi, Uttar Pradesh, India

Dhruv Kumar Amity Institute of Molecular Medicine & Stem Cell Research Lab, Amity University, Noida, India

James Eduardo Lago Londero Department of Biochemistry and Molecular Biology, Federal University of Santa Maria, Santa Maria, RS, Brazil

Himani Malhotra Department of Biotechnology, Lovely Professional University, Jalandhar, Punjab, India

Syed Faiz Mujtaba Department of Zoology, Shia P.G. College, Lucknow, India

Shama Parveen Molecular and Human Genetics Laboratory, Department of Zoology, University of Lucknow, Lucknow, India

Lipika Ray Pharmaceutics and Pharmacokinetics Division, CSIR-Central Drug Research Institute, Lucknow, Uttar Pradesh, India

Ratan Singh Ray Photobiology Laboratory, Systems Toxicology and Health Risk Assessment Group, CSIR-Indian Institute of Toxicology Research, Lucknow, India
Academy of Scientific and Innovative Research, New Delhi, India
Babu Banarasi Das University, Lucknow, India

André Passaglia Schuch Department of Biochemistry and Molecular Biology, Federal University of Santa Maria, Santa Maria, RS, Brazil

Abhishek Kumar Singh Amity Institute of Neuropsychology and Neurosciences, Amity University Uttar Pradesh, Noida, India

Bhupender Singh Department of Biotechnology, Lovely Professional University, Jalandhar, Punjab, India

Jyoti Singh Photobiology Laboratory, Systems Toxicology and Health Risk Assessment Group, CSIR-Indian Institute of Toxicology Research, Lucknow, India
Academy of Scientific and Innovative Research, New Delhi, India

Sandeep Singh Department of Biochemistry, University of Allahabad, Allahabad, India

Jitendera Sinha Amity Institute of Neuropsychology and Neurosciences (AINN), Amity University, Noida, India

Ajeet Kumar Srivastav Photobiology Laboratory, Systems Toxicology and Health Risk Assessment Group, CSIR-Indian Institute of Toxicology Research, Lucknow, India
Babu Banarasi Das University, Lucknow, India

Sushant Kumar Srivastava Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi, India

Garima Suman Department of Radio-Diagnosis, Tata Memorial Hospital, Mumbai, India

Shankar Suman Department of Microbiology & Immunology, School of Medicine, Meharry Medical College, School of Medicine, Nashville, TN, USA

Manish Kumar Tripathi Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology, (Banaras Hindu University), Varanasi, India

Shambhoo Sharan Tripathi Bioscience and Bioengineering Department, Indian Institute of Technology-Bombay, Mumbai, India

Atul Kumar Upadhyay Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab, India

Neera Yadav Molecular and Human Genetics Laboratory, Department of Zoology, University of Lucknow, Lucknow, India

Gonzalo Gurrea Ysasi Departamento de Construcciones Arquitectónicas, Universitat Politècnica de València, Valencia, Spain

Abbreviations

ECM	Extracellular matrix
HSPGs	Heparan sulfate proteoglycans
MMPs	Matrix metalloproteinases
TIMP	Tissue inhibitor of metalloproteinase
NCBI	National Centre for Biotechnology Information
KSCs	Keratinocyte stem cells
CPDs	Cyclobutane pyrimidine dimers
SCC	Squamous cell carcinoma
BCC	Basal cell carcinoma
MED	Minimum erythemal dose
NMSC	Nonmelanocytic skin cancers
BCC	Basal cell carcinoma
SCC	Squamous cell carcinoma
MDR	Multi-drug resistance
NCIA	National Cancer Institute of America
PDT	Photodynamic therapy
NGS	Next-generation sequencing

Skin Anatomy and Morphology

Neera Yadav, Shama Parveen, Shilpa Chakravarty, and Monisha Banerjee

Abstract

The skin as an outer covering of the body is the largest organ and is obligatory for the support and protection of internal organs to function properly. It varies in thickness depending on its site and role it performs. It contains many appendages and glands to maintain homeostasis and normal temperature of the body. It also contains pigments which act as sunscreen to protect against UV radiation and is the site of vitamin D synthesis which is necessary for normal growth. The skin is divided in many layers based on the cellular structure to communicate in a way so as to function effectively. The cells of the skin contain numerous connecting proteins for transfer of messages across them. Genetic disorders in some of these proteins may lead to serious diseases.

Keywords

Skin · Largest organ · Homeostasis · Protection · Dermis · Appendages

1 Introduction

The human skin or cutaneous covering is the external covering of the body. Its origin is ectodermal. It is the major tissue system of the integumentary system, consisting of the skin and the outer and inner linings of internal organs. The skin protects internal organs, bones, muscles, and other soft structures from harmful environmental agents, chemicals and pathogens, etc. It plays an important role in the synthesis

N. Yadav (✉) · S. Parveen · M. Banerjee
Molecular and Human Genetics Laboratory, Department of Zoology, University of Lucknow, Lucknow, India

S. Chakravarty
Biochemistry Department, University of Allahabad, Prayagraj, India

A. Dwivedi et al. (eds.), *Skin Aging & Cancer*,
https://doi.org/10.1007/978-981-13-2541-0_1

of vitamin D, sensation, and temperature regulation of the body. The skin also provides first line of defense from pathogens. The total surface area of the average adult human skin ranges from 1.5 m^2 to 2.0 m^2. The thickness of the skin ranges from 0.3 to several centimeters. The skin is thinnest at eyelids and thickest at soles and palms. The skin can have weight of about 20 kg. The human skin is usually covered with small hairs. On the basis of hairs, the skin may be hairy or non-hairy. The non-hairy skin is called the glabrous skin. The skin has a property to revive itself continuously; even the severely damaged skin can heal by forming scar tissue which may be faded. Skin, depending on the type of cell and tissues, is divided into different layers. Cells are stacked into multiple layers together with connective tissues to form different configurations within each layer. Deep into the skin, numerous blood capillaries are entrenched to provide proper vascularization. It is well equipped with sensory system. One centimeter square area of the skin typically contains 5 hairs, 6,000,000 cells, 5000 sense end organs, 15 sebaceous and 100 sweat glands, 200 pain sensors, 12 cold and 2 heat receptors, 400 cm nerve fibers, and 100 cm blood vessels. The skin of different locations has modifications according to the need of the site at which it is present. For example, soles have thick epidermis, the face is well furnished with sebaceous glands in bulk, and the scalp is roofed with thick layer of keratinized cells in the form of hairs. Typically, the skin is divided into the outermost *epidermis*, inner *dermis*, *basement membrane zone*, and *subcutaneous* tissue.

2 Epidermis

The word epidermis is made of two different words; i.e., epi means outer and derma means skin. Henceforth, the epidermis is the outermost surface of the skin which is in direct contact with the environment. The epidermis lacks vascular system as it has no blood vessels; this condition is known as *avascular*. It consists of keratinized, stratified squamous epithelial tissue. The epidermis of different locations has different thicknesses of epithelial cell layers, but commonly ranges from four to five. These grounds varied thickness of the epidermis at distinct sites. At distinctive sites such as soles and palms it can go up to 30 cell layers. The skin with up to four cell layers is designated as "thin" skin whereas, beyond it, skin is called "thick." Except soles and palms, all the skin is thin. On the basis of arrangement of cells, layers have been categorized into four types, viz., *stratum corneum*, *s. granulosum*, *s. spinosum*, and *s. basale* (from outside to inside).

2.1 Stratum Corneum

The outermost layer needs resurgence as it is directly pretentious by the environmental wear and tear. This perseverance can be achieved by stratum corneum, the outermost layer of the epidermis. It keeps continuous shedding of multilayer keratinocyte cells (10–30 cell thick) to maintain skin hale and hearty. Keratinocyte cells of the stratum corneum are hardened as a horn of an animal and, thereby, known as

"horny cell layer." As outermost cells grow older, soon they get damaged and become less active which results in sloughing off the dead cells to give space for new cells daily. It is an unremitting process which can take up to 28–50 days depending on the age of a person. The process of shedding slows down with the age of a person; as a result older people have rough and wrinkled skin.

2.2 The Stratum Granulosum

It is a 3–5-cell-thick layer which constructs the water-resistant barrier of the epidermis. It retains moisture of the lower epidermis by blocking passage of water and other water-soluble substances to underneath surfaces. Cells of this layer are flattened and rectangular or polygonal in shape containing asymmetrical granules of two types, viz., the *lamellar granules* and the *basophilic keratohyalin*. These granules secrete certain substances performing different functions within the epidermis. For example, lamellar granules secrete a lipid-rich substance that accumulates in extracellular matrix to hold cells tightly closed to each other and avoid water loss. The basophilic keratohyalin secretes keratin proteins *tonofilaments* and *filaggrin* which together form pre-keratin structures called tonofibrils. These structures play an important role in keratinization.

2.3 Stratum Lucidum

It is a layer of closely packed flattened dead cells which can be found only in palms or soles. It is found between stratum granulosum and stratum corneum layers of the epidermis. It helps providing stretching, water proofing, and reduction of friction in the skin. Its thickness varies in different parts of the body but is thickest at soles and palms (Fig. 1).

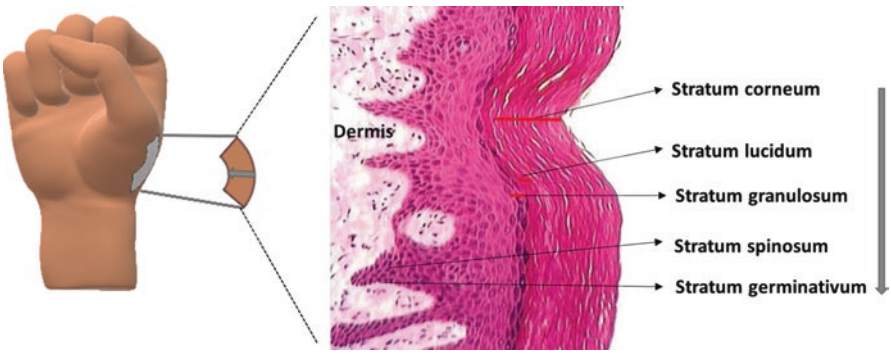

Fig. 1 Layers of epidermis

2.4 Stratum Spinosum

It is also known as prickle cell layer or spinous layer, located between the stratum granulosum and stratum basale of the epidermis. Cells of this layer have spiny appearance due to cringing of the microfilaments stuck between desmosomes. This layer consists of keratin-producing cells which produce fibrillar proteins called *cytokeratin*. Cytokeratin amassed to form *tonofibrils* within the cells which in turn form desmosomes (resilient connections between cells). Keratinization embarks in this layer only. There are several layers of polyhedral keratinocytes in it; the deepest endures mitosis to help in replacement of external hoary cells.

3 Stratum Basale

The spinous or prickle cell layer is located between the stratum granulosum and stratum basale of the epidermis. Cells of this layer are columinar or cuboidal in shape with a large nucleus. Cells are connected to the stratum spinosum through desmosome and hemidesmosome connections. Some of its cells act as stem cells to produce new cells to refurbish shedding epidermal portion, while the rest of the cells move outward and differentiate to get matured to form keratinocytes of the overlying layer, i.e., stratum spinosum. Cells of this layer undergo mitosis to form new cells to help rejuvenate the skin. Additionally, this layer consists of cells which perform vital roles in the skin, such as pigment-producing *melanocytes*, immune cells *Langerhans*, and touch receptors *Merkel cells*. Many skin problems including basal cell tumors and psoriasis arise in this very layer of epidermis.

3.1 Basement Membrane

The basement membrane layer is formed by different *ECM* (extracellular matrix) proteins like collagen, heparan sulfate, entactin, laminin, glycosaminoglycan, etc. These proteins are produced by keratinocyte cells in combination of fibronectin which is produced by dermal fibroblast. The basement membrane attaches with the basal side of epithelial cells via cellular receptors such as *integrin* and *dystroglycan*. The basement membrane also plays an important role in embryonic and organ morphogenesis.

4 Layers of Basement Membrane

4.1 Basal Lamina

It is made up of two precincts called the *lamina lucida* and *lamina densa*.

4.2 Lamina Lucida

It is a roughly 40-nm-wide (electron-dense) lamina densa of the basement membrane and electron-lucent zone flanked by the plasma membrane of the basal cells. It is composed of glycoprotein laminin.

4.3 Lamina Densa

The lamina densa is the part of the basement membrane zone sandwiched between the epidermis and dermis and is an electron-dense zone stuck between the lamina lucida and dermis. This zone is synthesized by the basal cells of the epidermis and is composed of anchoring fibrils made of type VII collagen, type IV collagen and dermal microfibrils, etc. It is a 20–50-nm-thick structure.

4.4 Lamina Reticularis

Lamina reticularis with the help of type VII collagen fibers (anchoring fibrils) is attached to the basal lamina and microfibrils (fibrillin). It is always toward the connective tissue and it is made by the connective tissue. It is mostly absent in muscle fibers, Schwann cells, and capillary endothelia. Collagen I fibers are found on the external side of the reticular lamina.

The function of the basal lamina varies according to the cell types. In different organs the organization of the basement membrane changes according to their function. In kidney glomerular, the basal lamina is not usual. A *lucent zone* is found on both sides of the lamina densa.

Lucent zones are categorized into two types, *lamina interna* and *lamina externa*.

The basement membrane of the cornea is known as *Descemet's membrane*. The basal lamina is much thicker in these membranes and the type IV collagen that is commonly found is replaced by type VIII collagen. The basal lamina of the neuromuscular junction has heparin sulfate proteoglycan (agrin) that helps in assembly of muscle of acetylcholine receptors in the junction of the plasma membrane. The epithelial cell's basal lamina helps in anchoring of epithelial cells which is also produced by these cells. When two basal laminae are joined to each other and there is no separation by reticular lamina, it is called the basement membrane. This type of structure is also found in some tissues.

It performs different functions in different cells according to location. In the skin, the epidermis to the dermis is bound tightly by the basement membrane at the *dermal epidermal junction* (DEJ-BM). DEJ-BM acts as an important permeability barrier that controls exchange of macromolecules. Restoration of skin's normal functional properties after wounding is accomplished by repair of the DEJ-BM during wound healing (Fisher and Rittie 2018).

Components of basement membrane – The basement membrane encompasses different types of protein-like heparan sulfate proteoglycans (HSPGs) and perlecan,

laminin (heterotrimeric), one or more of the variants of type IV collagens, and one or two nidogens. These component proteins form the basic structure of the basement membrane and play a role in the interaction of ligands. The differences in laminin and collagen isoforms result in differences in their assembly which affects their receptor binding and subsequent cross-linking properties, allowing variations in final structure, stability, and signaling. Laminins, perlecan, type IV collagens, and agrin are large macromolecules ranging from 75 nm to 400 nm. In typical thin basement membranes (50–100 nm thick), the basic components generally form a single molecular layer with the long axes of laminin and collagen parallel to the surface (Yurchenco 2011). Each laminin (400–800 kDa) is a heterotrimeric molecule consisting of one each of five *a*, four *b*, and three *g* subunits joined through a long coiled-coil domain (Parsons et al. 2002; Aumailley et al. 2005). Integrins are transmembrane heterodimeric receptors that mediate signaling initiated by ligand binding. They act bidirectionally and are modulated by the mechanical properties of the cell-ECM interface (reviewed in Berrier and Yamada 2007; Takagi 2007). Dystroglycan is part of a complex (dystrophin-glyco-protein complex, or DGC) that forms a link in a chain of bound proteins extending from laminins, agrins, and perlecan to a-dystroglycan to b-dystroglycan to dystrophin/utrophin to F-actin (reviewed in Barresi and Campbell 2006). Netrins (discovered as Unc6 in C. *elegans*) represent a family of secreted ECM proteins found both within and outside the central nervous system, affecting axonal guidance, interneuronal migration, vasculogenesis, and branching morphogenesis (reviewed in Yurchenco and Wadsworth 2004; Cirulli and Yebra 2007).

5 Functions

It provides support and help in anchoring of cells. It acts as a selective permeable barrier between tissues. It also helps in the work of cell division and instructs the growing process of cells during development and repair of tissue. It plays an important role in angiogenesis (development of new blood vessel).

In genetic disease related to the basement membrane, *congenital muscular dystrophies* are disease related to laminin disorders. In this disease there is a mutation in the *LAMA2* gene. It also arises from mutation in laminin receptors, i.e., integrin α7β1. Epidermolysis bullosa is a blistering disease. This splitting of the epidermal basement membrane and mutation are found in LAMA3, LAMB3, or LAMC2. This disease also results from keratin gene mutation. Elevated level of collagen VII is common in patient related to systemic sclerosis. Pierson's syndrome is caused by mutation in the *LAMB2* gene. *Bullous pemphigoid* is an autoimmune disease; immune system produces autoantibodies against the basement membrane (lamina lucida) between epidermis and dermis.

6 Basement Membrane Role in Cancer

The basement membrane provides a physical barrier to the stroma and epithelial cells. Proteins (glycoprotein and proteoglycan) by which formation of the basement membrane occurs act as a ligand for integrins and help in outside and inside signaling of cells. In cancerous cells the components of the basement membrane are modified. Reduced laminin levels are observed in cancerous cells. Activity of ECM proteases is inhibited by these proteins. In tumor cells the balance between ECM proteases and their inhibitor proteins is altered. MMPs (*matrix metalloproteinases*) are elevated and degrade ECM proteins. The level of collagen protein degradation is increased in metastatic cells.

7 Dermis

The skin is made up of mainly two layers of cells, innermost or lower layer known as dermis. The thickest layer of skin lies below the epidermis and above to the subcutaneous layer. Due to the presence of fibrous and elastic tissue, it protects the skin and provides flexibility. The dermis consists of sweat gland (regulates body temperature), blood vessels, lymph vessels, hair follicles, and sebum (an oily substance) that protect the skin from dryness. This component reaches at the skin surface through small openings of skin which act as pores. The dermis consists of two regions, *papillary dermis* and *reticular dermis*.

7.1 Papillary Dermis

It is found below epidermal junction, a thin layer made up of loose connective tissue. A component that includes collagen protein, elastic fibers, reticular fibers and capillaries, etc.

7.2 Reticular Dermis

The reticular dermis is the thickest and the deeper layer of the dermis is located just above the subcutaneous layer. It contains compact arrangement of connective tissue that includes mast cells, blood vessels, collagen fibers, fibroblasts, nerve endings, and lymphatics. A component of the dermis is surrounded by substances like mucopolysaccharides, chondroitin sulfate, and glycoprotein.

Cells and components are found in the dermis.

7.2.1 Fibroblast

These cells are responsible for the secretion of elastic fibers and procollagen. Procollagen is catalyzed by different enzymes and converts into collagen by formation of disulfide bond by cross-linking which creates strong layers.

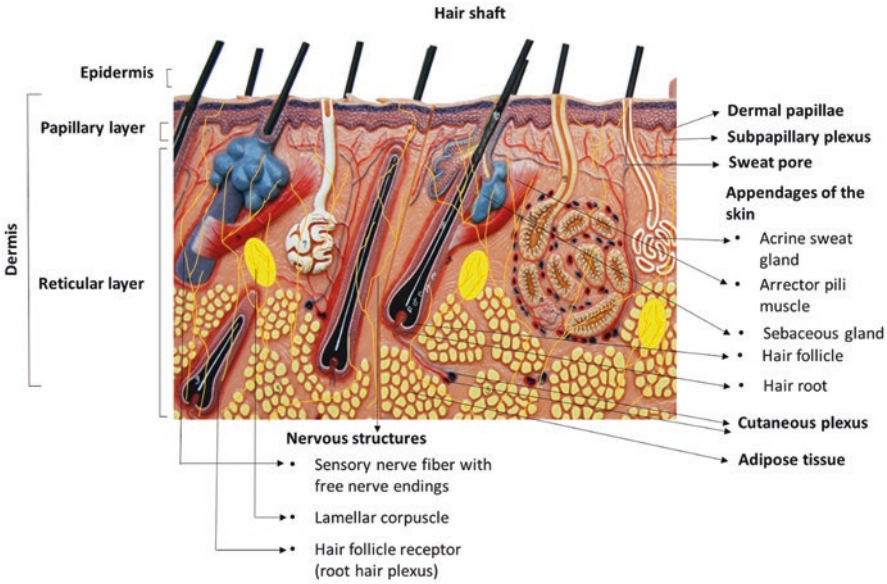

Fig. 2 Structure of dermis

7.2.2 Collagen

It comprises more than 60% weight of the dermis. Mostly type I collagens are found but less amount of type III is also present (Fig. 2).

7.2.3 Hair Follicles

Various types of hairs found on skin are produced by hair follicles. This hair not only plays a role in the appearance of a person but also helps in regulating body temperature, protects skin from injury, and inhibits sensation. A follicle also has stem cells which help in regrowing of damaged skin.

7.2.4 Elastic Fibers

It provides elasticity to the dermis but these fibers are present in very less amount (less than 1%v of the dermis). It also plays an important role to resist the skin from forces that can change the shape.

Mucopolysaccharide helps in diffusion of nutrient and waste product to other tissues by the binding of two types of fibers together.

8 Mast Cells

It releases histamine (present in granules) and some other chemicals in damaged condition.

9 Vascular Smooth Muscle Cells

It helps in maintaining the body temperature (*homeostasis*) and also responsible for dilation and contraction of blood vessels.

10 Sweat Glands

These are twisted structures found almost on our body and consist of two portions, *secretory section* and *excretory duct*.

Location of secretory portion is in the dermis but some time it is also present in the hypodermis (deeper layer of skin). Opening of secretory portion is present at very top and secretory duct (portion of secretion) moves from dermis to epidermis (top most layer), opens at skin surface.

11 Types of Sweat Gland

Eccrine glands
Apocrine glands

11.1 Eccrine Glands

Eccrine glands are also true sweat glands and play an important role in maintaining body temperature.

11.2 Apocrine Glands

Apocrine glands are found in places like armpits, scrotum, anus, and labia majora. Ducts of these glands open into hair follicles. These glands are not important for homeostasis, but play a role in body smells.

12 Oil (Sebaceous) Glands

These glands are present in most of the skin except the palm and feet. Sebaceous glands secrete oils. Their function is to secrete compound sebum that is a mixture of sebum-producing cells, a mixture of fatty substance, and epithelial cell debris. Opening of these ducts into upper part of hair follicle is known as infundibulum. Sebum acts as a lubricant for the skin and helps in preventing the excess evaporation of H_2O from the skin. Some chemicals are also present in sebum that kill bacteria and prevent their entry into the deeper layer of the skin.

13 Blood Supply in Dermis

Blood supply provides temperature regulation, network of arteries in subcutaneous layer, and the sub-papillary plexus in the dermis. The sub-papillary plexus helps in giving the pink color of the skin. A hemangioma that is dense collections of dilated blood capillaries is present in birthmarks.

13.1 Disorders Related to Dermis

1. Cutaneous atrophy – Striae, solar elastosis, white fibrous papulosis of the neck (Shimizu), lichen sclerosus et atrophicus (LA), Werner's syndrome, Rothmund-Thomson syndrome, progeria, and acrogeria
2. Dysplasia – Congenital ectodermal dysplasia

14 Functions

It provides the skin its flexibility and strength.

It provides nutrition to the skin and regulates temperature.

Sebum (secreted by sebaceous glands) acts as a barrier against pathogens.

Nerve endings are present in the dermis which can sense touch, pain, temperature, and pressure.

References

Aumailley, M., Bruckner-Tuderman, L., Carter, W. G., Deutzmann, R., Edgar, D., Ekblom, P., et al. (2005). A simplified laminin nomenclature. *Matrix Biology, 24*(5), 326–332.

Barresi, R., & Campbell, K. P. (2006). Dystroglycan: From biosynthesis to pathogenesis of human disease. *Journal of Cell Science, 119*(2), 199–207.

Berrier, A. L., & Yamada, K. M. (2007). Cell–matrix adhesion. *Journal of Cellular Physiology, 213*(3), 565–573.

Cirulli, V., & Yebra, M. (2007). Netrins: Beyond the brain. *Nature Reviews Molecular Cell Biology, 8*(4), 296.

Fisher, G., & Rittié, L. (2018). Restoration of the basement membrane after wounding: A hallmark of young human skin altered with aging. *Journal of Cell Communication and Signaling, 12*(1), 401–411. https://www.derm-hokudai.jp/shimizu-dermatology/pdf/18-01.pdf.

Parsons, M. J., Pollard, S. M., Feldman, B., Coutinho, P., Hirst, E. M., & Stemple, D. L. (2002). Zebrafish mutants identify an essential role for laminins in notochord formation. *Development, 129*(13), 3137–3146.

Takagi, J. (2007). Structural basis for ligand recognition by integrins. *Current Opinion in Cell Biology, 19*(5), 557–564.

Yurchenco, P. D. (2011). Basement membranes: Cell scaffoldings and signaling platforms. *Cold Spring Harbor Perspectives in Biology, 3*(2), a004911.

Yurchenco, P. D., & Wadsworth, W. G. (2004). Assembly and tissue functions of early embryonic laminins and netrins. *Current Opinion in Cell Biology, 16*(5), 572–579.

Understanding Cellular and Molecular Events of Skin Aging and Cancer: An Integrative Perspective

Bhupender Singh, Himani Malhotra, Dhruv Kumar, Syed Faiz Mujtaba, and Atul Kumar Upadhyay

Abstract

Aging of the skin is a gradual process, which is associated with changes in the appearance, characteristics, and function of the skin. Skin aging occurs through genetic, lifestyle, dietary, and environmental factors. Within the skin the production of collagen and elastin slows down, dead skin cells do not shed quickly, and the turnover of new skin cells decreases between the age group of 20 and 50. In addition to natural aging premature aging can also result which is due to sunlight exposure, chemical exposure, or other environmental pollutions. Ultraviolet radiation in sunlight damages the elastin and collagen fibers in the skin, which ultimately results in wrinkling of the skin at early stages of life. UV light induces approximately 99% of non-melanoma and 95% of melanoma-type skin cancers in humans. Excessive UV exposure produces genetic mutation that can lead to skin cancer. Skin carcinogenesis by DNA damage is considered a predominant paradigm for UV toxicity. Exposure to UV radiation can activate many oncogenes, which leads to skin cancer. Initiation and progression of skin carcinogenesis mediated by UV radiation involve complex pathways including those of apoptosis, proliferation, autophagy, DNA repair, metabolism, and inflammation. PTEN (phosphatase tensin homolog) is well established as a tumor suppressor gene that induces apoptosis and reduces cell proliferation by inhibition of the

B. Singh · H. Malhotra
Department of Biotechnology, Lovely Professional University, Jalandhar, Punjab, India

D. Kumar
Amity Institute of Molecular Medicine & Stem Cell Research Lab, Amity University, Noida, India

S. F. Mujtaba
Department of Zoology, Shia P.G. College, Lucknow, India

A. K. Upadhyay (✉)
Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab, India

A. Dwivedi et al. (eds.), *Skin Aging & Cancer*,
https://doi.org/10.1007/978-981-13-2541-0_2

P13K/AKT pathway. p53 gene present in humans is being positioned on chromosome number 17 and mutations analyzed in the tumor suppressor gene p53 are one of prior genetic events which lead to the development of cancer in the skin due to exposure to UV rays.

In this chapter we have discussed the mechanism of aging and skin cancer as an integrative phenomenon. We have also discussed briefly regarding the molecular biology methods and computational approaches to study the skin cancer and aging in a greater depth.

Keywords
Skin cancer · Aging · Molecular biology · Bioinformatics and melanoma

1 Introduction

Aging can be defined as the collection of advancements in the body with progress of time which leads to enhanced likelihood of disease occurring and demise. In other words aging is the threshold level of the functions and structures which occurs inside an organism throughout its growth and development. A famous American gerontologist named Bernard Strehler put forward aging as a mechanism having four features, which are: (A) universal, present in all organisms of a species at varying pace; (B) intrinsic, agents of aging that must be present within, which do not rely on external elements; (C) progressive, alteration caused by aging that must occur during all advancement of life of an organism; and (D) deleterious, processes associated with aging that must have negative impact on an individual (Viña et al. 2007).

With reference to 'free radical theory of aging' postulated by Denham Harman, he states that usual production of ROS (reactive oxygen species) along with cellular components regulates alterations related to aging. The skin serves as a brilliant and model organ to perform research on aging. Skin aging is attributed to atrophy, emergence of wrinkles, decreased tractile power, and diminished bruise curing. In response to ROS, human skin contains collection of antioxidants and enzyme system. These antioxidants and enzyme system guard the cells from harmful outcomes of free radicals. Antioxidant compounds named as vitamin A, C, and E decrease the rate of aging progression by two means. The first way is to avoid oxidation of biological molecules by free radicals and second is by decreasing the production of free radicals and suppressing the ROS which were formed previously. Apart from this superoxide dismutase, catalase, and glutathione biosynthesis enzymes guard the tissues from free radicals. The degree of antioxidants and its enzymes is decreased by internal and external contributors which are age and environmental factors. Primary antioxidants prevent oxidation by chain termination step. This reaction involves migration of a proton to free radical species. Glutathione, alpha-tocopherol, vitamin

E, and its derived products and ascorbic acid (vitamin C) are some primary antioxidants. Glutathione and ascorbic acid are water soluble and fat soluble, respectively. Membrane-bound antioxidants are vitamin E and ubiquinols, respectively. Secondary antioxidants are used in synergy and they prevent destruction of primary antioxidants. Lipoic acid is a secondary antioxidant in the human skin which is water as well as lipid soluble. It also acts as an important cofactor for various enzyme systems (Harman 1956; Pinnell 2003).

In this chapter we will be discussing about various cellular and molecular events associated with progress of skin aging and cancer.

2 Cellular Senescence and Skin Aging

The organisms which have multifarious body organization consist of mitotic and postmitotic cells. The cells which have the ability to undergo cell division are called mitotic cells, while the cells with no cell division potential are termed as postmitotic cells. Epithelial and stromal cells of the skin are examples of mitotic cells whereas grown-up muscle and nerve and fat cells are some examples of postmitotic cells. As mitotic and postmitotic cells have contrast in their cell division, there is possibility that they possess dissimilar aging process. There are several various aspects of aging; one can be due to altered gene expression of mitotic cells and injury in the postmitotic cells. The frequency of cell division cycle in a typical cell prior to its senescence relies on factors such as the type of species, age, nature of cell, and genomic makeup of the contributor. For example, in the case of fetal or neonatal human cells, around 60–80 divisions take place. It has been found by the researchers that cells have the ability to intellect frequency of divisions finished mainly by extent of their telomere (Stanulis-Praeger 1987).

Seizing of cell multiplication is a characteristic of replicative senescence with reference to repression of tumor. In addition to this senescent cells also express two physical makeup alterations. First alteration is the senescent cells overcome apoptosis mechanism. This alteration is achieved by sustaining bcl-2 protein. This protein protects the cell from mortality. This characteristic feature of senescent cell to overcome apoptosis emphasizes on the peculiarity between cell death and cell senescence. This characteristic elaborates the collection of senescent cell with age in skin and other body tissues. In a study a biomarker named β-galactosidase which was present in only senescent (not even in presenescent cells) cells of fibroblasts and keratinocyte was identified. This biomarker was not present in inactive fibroblasts and keratinocyte evolved at terminal. By taking the skin sample of human at different ages, they found that the concentration of this biomarker in dermal fibroblast and epidermal keratinocyte was increasing in accordance to age (aged samples were showing high concentration). With this they confirmed the presence and collection of senescent cells with age in vivo. Secondly, senescent cell display alteration in the function arises by differentiation. For example, in a study it was found that

senescent human fibroblasts and endothelial cells showed elevated levels of cytokine interleukin-1α. This cytokine is responsible for hindrance of endothelial cell growth in in vitro condition. In another work it was found that senescent human endothelial cells elevated level of ICAM (intercellular adhesion molecule), which is a cell-type based adhesion molecule. This overexpression partially regulates increased connection among monoblast U937 and senescent endothelial cells. Moreover in senescent mammary epithelial cells were found to be showing elevated expression of retinoic acid receptor (β-isoform) (West et al. 1989).

From several studies it is evident that deposited senescent cells (skin-type) directly link to functional characteristics of skin aging. In other words we can say that replicative copies of senescent cells alter the regular tissue role in the organ. This statement gets validated in the case of skin. In a separate study it was found that presenescent dermal fibroblast showed relatively stumpy expression of matrix metalloproteinases – collagenase and stromelysin. Matrix metalloproteinases account for degradation of extracellular matrix proteins. Further it was also found that these presenescent dermal fibroblasts were overexpressing tissue inhibitor of metalloproteinase 1 and 3 (TIMP 1 and 3). From this they made it evident that after senescence TIMP 1 and 3 expression elevates and collagenase and stromelysin (matrix metalloproteinase) expression drops off. Thermal fibroblasts presented as a model to study how replicative senescence plays a central role in shift of functional characteristics. Replicative senescence alters the functional character of matrix metalloproteinase by shifting its expression from matrix fabricating to matrix demeaning. The decrease and disconfirmation of dermal collagen are expression patterns of skin aging. From these studies it could be concluded that there is strong relation of deposition of senescent cells with dermal atrophy which arise with age (Maier et al. 1993; Swisshelm et al. 1994).

3 Solar Radiations and Skin Aging

In this part we will be discussing the impact of UV (ultraviolet), visible region, and IR (infrared) radiation on skin aging. The UV region falls under the wavelength of 290–400 nm, visible region comes under 400–700 nm wavelength, and IR region starts after 800 nm wavelength. Now the role of an individual region in skin aging will be highlighted.

3.1 UV Region Impact on Skin Aging

The UV region of the solar radiation consists of UVA, UVB, and UVC. UVC region has wavelength range 100–290 nm, UVB has wavelength 290–320 nm, and UVA comprises of wavelength 320–400 nm. Decidedly lethal radiation among these is of UVC. It is trapped by the ozone layer, humidity in ambience, and prevents its entry in earth's shell. Free radicals are responsible for around half of the damage by UV and remaining damage fulfilled by straight cell injury. Cellular photosensitization leads to oxidation mechanisms which further regulates the UV-mediated reactions

at a cellular level. The UV radiation is taken up by cellular constituents such as nucleic acid, urocanic acid, aromatic amino acids (tryptophan and tyrosine), quinones, flavins, porphyrins, and NADH. Further this action leads to production of hydrogen peroxide, hydroxyl, and oxygen-free radicals (Trautinger 2001).

The radiation is primarily taken up by cellular constituents in UVB region. UVB is unswervingly taken up by deoxyribose nucleic acid (DNA) of epidermis cells. This take-up leads to the formation of distinctive cyclobutane pyrimidine dimers and pyrimidine cross-linked dimers. This pyrimidine dimer further regulates skin melanogenesis. This mechanism protects the skin from additional UV injury. This injury in DNA prevents the transcription and thus activation of p53 proteins. Further it leads to keratinocyte apoptosis in epidermal cells which forms 'suntan' cells. Extended exposure of the radiation is capable of repressing p53 intervened apoptosis which reflects in collection of injured cells. This repression becomes more lethal when it contributes to activation of mutagenesis and photo-carcinogenesis (Fisher et al. 2002).

On the other side UVA promotes photoaging by targeting dermal matrix. ROS interfere with TF (transcription factor) activator protein 1 by increasing its response which results in increased translation of matrix metalloproteinases (MMP). Matrix metalloproteinases are responsible for disruption of collagen. Various matrix metalloproteinases like MMP-1 (interstitial collagenase), MMP-3 (stromelysin), and MMP-9 (gelatinase) are translated. Leukocytes, keratinocytes, and macrophages start the degradation of dermal matrix proteins. Injury in collagen, GAGs (glycosaminoglycans), and elastin reflects physical characters of photoaging by rooting elastosis and wrinkles. In addition, TF activator protein 1 blocks translation of collagen in dermal cells by retarding transforming growth factor-β and thus expression of type I and type III procollagen is downregulated (Sklar et al. 2013).

Sensitive UVB radiation results in sunburn, which after 6–24 h rises at its threshold. Sunburn results in ruddiness and puffiness because of liberation of proinflammatory moderators and vasodilation of dermis blood vessel. On the other hand, impermanent exposure of UVA results in immediate pigment darkening as a result of photooxidation of melanin and its reorganization in keratinocytes. Immediate pigment darkening disappears swiftly but, when exposure lasts more than 10 Jcm^{-2}, results in persistent pigment darkening. Long-standing exposures of UVB result in delayed tanning response which arises because of initiation of melanogenesis (Kollias and Baqer 1984).

3.2 Visible Region Impact on Skin Aging

Visible region of light falls under the wavelength of 400–700 nm. This region of light is stated as non-detrimental. It contributes to around 40% of solar rays which falls on earth's shell. Visible light may account for situations like chronic actinic dermatitis, solar urticaria, porphyrias, erythema, initiation of pigment formation, and phototoxic and photoallergic skin responses. In a study it was found that visible irradiation on skin in vivo has the ability to initiate skin pigment formation and its survival rate is around 10 weeks. Another study reveals that visible light of wavelength till 470 nm was able to persuade immediate pigment darkening. Heightened

response of immediate pigment darkening ranges from 300 nm to 500 nm. Apart from this visible region also accounts for erythema, which was showing higher response in individuals with darker skin as compared to the one with light skin-type. The heightened response in darker skin positively correlates with elevated levels of color-giving compounds such as melanin. Melanin soaks light-producing heat leading to dilatation of blood vessels and accounts for erythema (Bernstein et al. 2004).

Visible light promotes skin damage partially by oxidative stress. Taking into account in vitro approaches it was found that in skin cells the incidence of visible light produces micronuclei devoid of cyclobutane pyrimidine dimers. This is further linked with initiation of oxidative DNA injury in skin cells. The threshold of wavelength causing DNA damage varies from 400 nm to 450 nm. The exposure of human skin with visible light starts the production of ROS, matrix metalloproteinase-1, matrix metalloproteinase-9, and pro-inflammatory cytokines (IL-1α, IL-1 receptor antagonist, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor). With the help of clinical models, incidence of visible light leads to heightened response of free radicals (produced as a result of oxidative stress). Visible light incidence also produces hydrogen peroxide which depends on dosage. In addition to this, treating the skin cells with antioxidants prior to visible light exposure inhibits the formation of free radicals. Moreover visible light exposure initiates EGFR (epidermal growth factor receptor)/ERK (extracellular signal-regulated kinase) pathway in keratinocytes (Liebel et al. 2012).

3.3 IR (Infrared) Region on Skin Aging

IR region of solar radiation falls under the range of 800nm–1 mm. It is further categorized into IR-A, IR-B, and IR-C. The wavelength of IR-A, IR-B, and IR-C comes under 700–1400 nm, 1400–3000 nm, and 3000 nm–1 mm, respectively. It contributes to around 50% of solar rays which falls on earth's shell. The uninterrupted exposure of sunrays to human skin elevates skin temperature to around 40 °C. The consumed IR is transformed into heat. Elevation in skin temperature depends on skin tone; that is, the darker the skin the higher will be the skin temperature. Greater than 65% of IR-A has the ability to reach the dermis and subcutaneous skin devoid of elevating skin temperature. IR-B and IR-C only elevate the skin temperature without entering beneath skin (Cho et al. 2009).

Light tone skin-type is more prone to IR-A as it leads to decrease in collagen production as well as elevated translation of matrix metalloproteinase-1 and matrix metalloproteinase-3. On the other hand, dark skin tone has least impact of IR-A on collagen production and matrix metalloproteinases as they trigger melanin synthesis. Disturbance of collagen balance in extracellular matrix of dermis is mediated by: lowering the new collagen formation and elevating translation of matrix metalloproteinase-1 which is responsible for disruption of collagen. IR-A-mediated translation of matrix metalloproteinase-1 is regulated by synthesis of intracellular ROS. It was found that IR-A radiation to average skin tone in vivo condition increased the transcription of matrix metalloproteinase-1 by 3–4 times. On exposure of IR matrix

metalloproteinase-1 translation was elevated by stimulation of mitogen-activated protein kinase signaling pathways (Schieke et al. 2003). IR-A-stimulated mRNA of human skin fibroblasts was studied. With the help of microarray examination 599 mRNA were found to be responsive on IR-A exposure. IR-A-induced genes belong to stress signaling, extracellular matrix, calcium homeostasis, and apoptosis. In real-time PCR it was found that 13 genes native to above-mentioned classes were responsive to IR-A radiation. With the help of chemical inhibitors it was demonstrated that ERK1/2, p38, JNK, P13K/AKT, STAT3, IL-6, and calcium-mediated signaling pathways were effectively indulged in IR-A-induced gene reaction. Further it was found that mainly mitochondrial ROS were involved in it while non-mitochondrial ROS were involved marginally (Calles et al. 2010).

3.4 Impact of Ozone on Skin Aging

Ozone is a three-oxygen-containing molecule denoted by 0_3 and is among lethal atmosphere compounds which compromise with human health. The top layer of human epidermis is targeted by the ozone, causing oxidation. Usually mean quantity of ozone in troposphere is below 0.08 parts per million while as in stratosphere its mean quantity is 10 parts per million. The amount of ozone over past three decades has elevated to 0.8 parts per million due to release of nitrogen oxides, methane, carbon monoxide, and sulfur-containing compounds. Ozone is unable to enter the skin but its lethal effects are triggered by oxidative stress which arises as a result of lipid peroxidation substances. Persistent irradiation of elevated levels of ozone in atmosphere leads to reduction of antioxidants and oxidation of lipids and proteins in the stratum corneum (top most layer of skin). Ozone damages lysine, cysteine, and histidine amino acids of protein and by production of 4-hydroxynonenal-protein (by addition reaction) lipids of skin are damaged. The impact of ozone on keratin cells maintained under lab conditions was observed. Ozone initiates the translation of pro-inflammatory cytokine IL-8 in skin. Apart from this, ozone in skin triggers keratin-10, PCNA (proliferating cell nuclear antigen) gene, Hsp (heat shock proteins), and markers of pro-inflammation – COX (cyclooxygenase)-2 and iNOS (inducible nitric oxide synthase). Ozone suppresses the transcription factor NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells). NF-kB is required for transcription of pro-inflammatory cytokines and contributes majorly for ozone-mediated skin inflammation. Introducing ozone to cells leads to amount-reliant elevation in transcription factor p65 subunit, which serves as an indicator of NF-kB initiation. NF-kB involves primarily in modulating cell reactions accountable for apoptosis signaling pathways, tumor promotion, metastasis, angiogenesis, cell proliferation, and inflammation. In vivo research confirms the impact of ozone in skin injury. The incidence of ozone elevated the expression of 4-hydroxynonenal and 8-iso-prostaglandin $F_2\alpha$ in epidermis consolidating the connection of ozone with lipids of skin. The upregulation of COX-2 and matrix metalloproteinase-9 was subsequently triggered by enhancement in NF-kB p65 expression on incidence of ozone. This also resulted in remarkable reduction in collagen-I and collagen-III (Valacchi et al. 2005).

4 Impact of AGEs (Advanced Glycation End Products) on Skin Aging

Glycation is defined as the reaction among sugars having free aldehyde or ketone group, proteins, lipids, and nucleic acids and is mediated without the application of enzymes. Some sugars having free aldehyde or ketone group are glucose, galactose, fructose, and xylose. Production of AGEs starts with Maillard reaction in which 'C=O (carbonyl)' group of sugars such as glucose establishes reaction with free amino group of mainly basic amino acids such as lysine or arginine, resulting in formation of unstable Schiff base. Even though Amadori product and Schiff bases are results of two-sided reactions, they can also react nonreversibly with amino acid dregs of proteins and comprise protein adducts. Optionally by oxidation, dehydration, polymerization, and oxidation break, various AGEs are formed. Various AGEs generally found in skin are glyoxal, methylglyoxal, GOLD (glyoxal-lysine dimer), and MOLD (methylglyoxal-lysine dimer). AGEs are consumed by food or generated by the organism's metabolism at small extent, though the rate of metabolically generated AGEs is elevated in diabetic individuals. Factors such as diet and smoking persuade speed of AGE production. The amount of AGEs in an individual is determined by the speed of AGE production as well as speed of their clearance. Various cells have formulated pathways for withdrawing lump of AGEs. Glyoxalase arrangement, which consists of glyoxalases I and II, has important function in protection from glycation (Fleming et al. 2011).

Aggregation of AGEs in dermis is a characteristic of skin aging. Collection of AGEs has been found throughout aging and diabetes in different tissues such as articular collagen. Skin autofluorescence linked with glycation displays relation with increasing age when detected in numerous fit individuals. Commonly, collection of AGEs is influenced by life span of proteins; that is, one with longer life span is considered to be primarily altered by glycation. With the progress of aging collagen types I and IV (life span of 10 years) and various other prolonged life spans, proteins such as fibronectin are altered by glycation. Glycation of collagen was first detected at 20 years of age. The aggregating speed of glycation annually is around 3.7%, which peaks up to 30–50% at 80 years span. Under lab conditions AGEs exhibit to alter the role of dermal cells. With the involvement of RAGE (receptor for AGE) and its association with induction of NF-kB and caspases, AGEs were responsible for reduction in cell multiplication and boosting programmed cell death in human skin fibroblasts. In keratin cells AGEs were responsible for reduction in cell feasibility and emigration and trigger the manifestation of pro-inflammatory moderators. In addition to this, in lab conditions AGEs are capable of activating premature senescence in human skin cells (fibroblasts) and keratin cells. With the activation of matrix metalloproteinases, the translation of collagen and extracellular matrix proteins was reduced. The epidermal growth factor receptor is responsible for regulating cell multiplication, differentiation, motility, and endurance. AGEs like glyoxal and methylglyoxal alter the signaling of epidermal growth factor receptor by generation of epidermal growth factor receptor network, further inhibiting phosphorylation and altering induction of phospholipase C and extracellular

signal-regulated kinases. There is evidence that glycation of basic fibroblast growth factor restricts its cellular activity (Dunn et al. 1991; Zhu et al. 2012).

5 Progeria Syndromes to Comprehend Skin Aging

Progeria syndromes are categorized under early aging syndromes. Focused study on these syndromes can help in comprehending important means which helps in regulation of aging. HGPS (Hutchinson-Gilford progeria syndrome) is exceptional genetically associated condition of early aging. This syndrome is a characteristic of scleroderma-type skin alterations, bone malfunctioning, hair loss, shortage of fat below epidermis, hindrance in growth, and inflexibility at connection between two bones. HGPS suffering individual's normal survival is for 13 years. HGPS individuals on the whole decease from atherosclerosis. HGPS condition results from change in one DNA base (GGC to GGT in exon 11 of lamin A) which results in expression of progerin protein in which 50 amino acids are removed. Astonishingly, the finding of progerin in usual cells implies means of progeria in general aging process. Progerin is found richly in aged individuals and late passage skin cells. The mechanism which leads to accumulation of progerin with age is found in skin projection, extending to cross-linked skin network with age, and some keratin cells (at their final differentiation stage) in mature individuals validate skin as ideal representative for human aging. Skin keratin cells of progeria-suffering individual show decreased intensity of CD34 and α-6 integrin (stem cell markers). Skin fibroblast of HGPS individual shows genetic faults like change in gene expression, nuclear bulging, disarrangement of elemental heterochromatin, impaired stem cell, enhanced damage to DNA, cellular senescence, and elevated $p16^{INK4A}$ (tumor suppressor protein) (DeBusk 1972; Rosengardten et al. 2011).

6 Implication of Biological Databases and Computational Tools to Perceive Skin Aging

Bioinformatics databases and various computational tools offer a wide array of options to make significant understanding in the case of skin aging. In study (Glass et al. 2013) the change in expression of genes associated with skin aging was analyzed. The aim was to find out the key pathways and genes linked aging in skin. Expression data obtained from Illumina probe was verified by using NCBI build 36 genome. *NCBI* (National Center for Biotechnology Information) (https://www.ncbi.nlm.nih.gov/) with the help of information technology aids in perceiving basic course regulating genetic and cellular level during the ailment and well-being. With the help of this resource only distinctively equivalent Illumina read was selected for further analysis. After this *IMPUTE* software was used to perform the imputation of undetected genetic constitution (Marchini et al. 2007). With the help of *eQTL (expression quantitative trait locus) mapping* in skin 2796 eQTL were determined (Glass et al. 2013). eQTL are genomic alternatives which change gene expression.

It is used to find the association among genetic variation and expression forms. Further *Ime4* (Bates et al. n.d.), a linear mixed model, was used and with the help of AIC (Akaike information criterion) top model which gives details regarding source of alteration in individual gene translation level was employed (Maser and DePinho 2002). At last it was found that noteworthy part of alteration in gene translation level associates with aging and these genes have impact in cell division control, senescence, apoptosis, and oncogenesis. The whole microarray profiling document can be accessed at *ArrayExpress* (http://www.ebi.ac.uk/arrayexpress) *database*, (Kolesnikov et al. 2014) archived under the ID- E-TABM-1140. With the help of computational tools and biological databases, the understanding at gene level can be made possible to gain insightful information in skin aging.

7 Cellular and Molecular Events: Skin Cancer

Mutations occurring among genes label the cell as cancerous cells. Skin cancer is one of the predominant cancers occurring among people; with the increase in number of patients suffering from skin cancer it has become a matter of great concern. One of the major causes of skin carcinoma is the extensive exposure of UV radiations to skin, although ozone present in stratospheric region leads to blockage of UVC (below 280 nm) radiations and also some segment of UVB rays (ranging from 280 nm to 290 nm) from reaching the surface of the earth, but some UVB (radiations in range 290–315 nm) and UVA (in range of 315–400 nm) rays arrive at the surface of the earth and cause damage to DNA, swelling and skin redness, peeling, variations in gene, immunosuppression due to swelling, and also skin cancer. UV radiations have an acute effect which encompasses damaging of DNA, lipid peroxidation, and protein cross-linking peroxidation; with increase in the number of exposure of UV radiations to skin, cancer occurs. Exposure to UVB radiation leads to formation of cyclobutane-type pyrimidine dimers and pyrimidine pyrimidone photoproducts. UV radiation persuades primarily C → T and CC → TT transitions at the di-pyrimidine sequences. These two transition variations are known to occur through the semiconservative type of replication of the DNA and wrong incorporation of A residues at noninstructional positions occurs, which is as per the A rule: DNA polymerase inserts A residues by default when it encounters abrasion in the template DNA that cannot be interpreted by it. High levels of p53 are expressed on DNA damage that causes the arrest of cell cycle, which further causes activation of repair pathways of cells to remove DNA abrasion that exists before the start of synthesis and mitosis of DNA.

Cancer due to UV radiation often leads to deactivation of tumor suppressor genes or the high activation of proto-oncogenes. Extensive exposure to UV radiations leads to the impairment of apoptosis (cell death) which causes divergent expansion of epidermal cell that produces keratin known as keratinocytes, including damaging of DNA, attainment of mutations in p53 gene, and inactivation of Fas receptor-Fas ligand interaction, which further leads to the start of skin carcinogen. Mutation in p53 gene by epidermal keratinocytes has a central role in skin carcinogenesis and in

Table 1 Tumor suppressor and oncogenes involved in skin cancer

S. No	Gene name	Function	Location	Type
1	p53	Involved in cell cycle arrest on DNA damage, activate proteins involved in DNA damage, initiate apoptosis if DNA damage is not repaired and also involved in aging	Nucleus of cell	Tumor suppressor gene
2	Patched	Role in hedgehog signaling pathway, cell growth and signal transduction pathway	12-pass trans-membrane protein	Tumor suppressor gene
3	Fas/FasL	Regulation of immune system, involved in apoptosis	Trans-membrane protein	Fas gene expression act as oncogene
4	Ras (H-, K-, N-)	Involved in cell growth, gene regulation and survival, cell transduction pathway	Cell membrane	Oncogene
5	Smoothened	Role in patched and hedgehog signaling pathway	Trans-membrane protein	Oncogene

skin exposed to sun and, which seems to be normal, thousands of p53 mutant cell clones are found. Due to long duration exposure to sun UV radiations, deactivation of interaction in Fas receptor and Fas ligand, and also p*53* mutations accumulated in the skin, cancer occurs (Brash et al. 1991; Ouhtit et al. 2000). Other tumor suppressors and oncogenes, which have a role in causing cancer in skin, have been discussed in Table 1.

8 Oncogenes and Tumor Suppressor Genes Causing Skin Cancer

There are several genes reported to be involved in the suppression of oncogenes and tumors in human. Few of these genes are discussed in this section as follows.

8.1 p53 Gene

p53 gene present in humans is being positioned on chromosome number 17 and consists of 11 exons comprising 20 kilobases. Mutations analyzed in the tumor suppressor gene p53 are one of prior genetic events, which lead to the development of cancer in skin due to exposure to UV rays. Mutations in p53 gene occur with great frequency causing premalignant solar keratosis and these mutations are considered to be the cause for squamous cell carcinomas (SCCs); for example, a study conducted for solar keratosis or actinic keratosis by Ziegler et al. gives the information that 66% of the lesions had mutations in p53 gene and C → T transition mutation was found in high proportion (23/35) (Ziegler et al. 1994).

When DNA damage occurs due to mutations caused by exposure to UV rays, tumor suppressor protein p53 leads to the cell cycle arrest of damaged DNA in G1 phase, so that repair of damage DNA could occur before its replication in S phase can be concluded. This causes the activation of p21 protein by p53 tumor suppressor protein; p21 further causes inactivation of complex CdK-cyclin by making complex Cdk2/A or Cdk2/E, cyclin/proliferating cell nuclear antigen/Waf1. Because of this complex formation, hypo-phosphorylation of retinoblastoma occurs, which further conducts release of E2F as it is required for initiation of synthesis of DNA formation.

p53 gene also causes apoptosis by upregulation of expression of proteins that are IGF-BP3, Fas, and BP3 proteins and also downregulation of expression of IGF-1R, IGFII, and Bcl2 proteins. The other pathway for apoptosis involves binding of p53 to other proteins of cells which are involved in synthesis of DNA like replicating protein antigen (RPA), proteins involved in mechanism of repair including group B (XPB) and D (XPD) DNA helicases of xeroderma pigmentosum, topoisomerase I, TFIIH, and p62 proteins (Dutta et al. 1993).

High exposure to UV radiations induces mutations in p53, which inactivates the functioning of p53 and causes skin carcinoma. Mutation of p53 protein is found to be at a high frequency in *Homo sapiens*. Cutaneous squamous cell carcinomas (SCCs) occur 90% in humans and basal cell carcinomas (BCCs) occur 56% among humans and they contain unique mutations at sites of di-pyrimidine; also studies found that mutations in p53 appeared in precancerous actinic keratosis and in damaged skin due to sun exposure. CT or CCTT base mutations at di-pyrimidine sites are mutations which occurred more frequently due to UV radiations and further cause skin cancer and SCC, sarcoma, and BCC which are types of tumor; 60% of the variations detected to be as mutations in p53 are CCTT transitions and sites where they occurred are identified as already known hotspots. Due to this distinctive quality of CCTT transitional mutations, they are also known as UV "signature mutations" (Dumaz et al. 1993; Hutchinson 1994).

Appearance of mutations in the tumor suppressor gene p53 is an advance genetic event for the growth of UV-induced cancers in skin. UV-induced skin cancers occurring in mouse have C → T transitional mutations and mutations occurring in *p53 protein are 70%*, although only 10–20% of CC → TT mutations have been detected. Interestingly, mutations in p53 were detected in the first week of persistent exposure of UV irradiations, and further the mutational frequency reached to 80–90% by 4–8 weeks (Dumaz et al. 1993). However studies have analyzed that mutation in p53 occurs well earlier the countenance of skin cancer.

p53 mutations have been appeared in almost one in every two cases occurring in cancer. All these mutations, their location, and frequencies have been listed and are known. By using these listed mutations of p53 a theoretical method has been validated which will further support in analysis of mutagenic regions of p53. Master code scores were finalized by analyzing 393 codons of p53, by using 61 value for these codons which are finalized from genetic code. Codons having scores near to 1 deduced to be conserved regions whereas codons having scores near to 61 deduced to be highly mutagenic regions.

8.2 Patched Gene

Development of tumor is a very complicated process that inferred either immediately or due to exposure to genotoxic stress. However, with the discovery of *ptc* gene, a gene involved in the hedgehog signaling pathway of development of embryo and also known to be as a tumor suppressor gene has given new insight into study of biology of tumor. Protein of *ptc* gene consists of 1286 amino acids with molecular weight of 143 kilodaltons (kDa) and is also predicted as an integral membrane protein (Hutchinson 1994). Homology of *Patched* gene present in *Drosophila* has been identified in *Homo sapiens* for causing nevoid basal cell carcinoma syndrome (NBCCS), an autosomal dominant disorder. The *ptc* gene opposes the function of *hedgehog (hh)* gene by repressing the cell growth and differentiation. The *hedgehog* genes are found to be activated untypically in patched mutated gene mutants and cell growth occurs abnormally when hedgehog gene signaling goes uncontrollable. Studies of NBCCS (nevoid basal cell carcinoma signal) patients have shown that mutations including genomic and sporadic were detected in the ptc gene, which are the utmost cause of cell NBCCS. *ptc* is a tumor suppressor gene which is being suggested from the number, distribution, and early out start of BCCs and mutations occurring in this gene cause skin basal carcinomas (Chen and Struhl 1996).

A polymorphism found in ptc gene which is C 3 T at nucleotide 2161 is an absolute polymorphism of the ptc gene. Most of the mutations detected in ptc gene which are 79% are found to be UV induced; all the 79% mutations are positioned at bi-pyrimidine sites. All these mutations occurred due to the absence of DNA repair mechanism of UV-exposed skin, which causes DNA lesions, which include mostly cyclobutane pyrimidine dimers and 6–4 pyrimidine-pyrimidone. So unrepaired DNA which occurred due to the long duration exposure to UV rays causes mutations in ptc gene. Fifteen out of 17 of the mutations, which are mainly substitutions found to be present in our xeroderma pigmentosum, are placed in the non-transcribed region of the strand of the ptc gene.

ptc gene can also act as an oncogene, as Hras mutations are observed in overexpression of *ptc* gene, and this further causes development of squamous carcinoma. Mutations in *ptc* gene cause abnormal expression or inactivation of genes involved in the SHH signaling pathway, finally leading to drawback in development, and result in cancerous phenotype. Mutations in the human *ptc* gene are responsible for NBCCS (nevoid basal cell carcinoma syndrome), distinguished by different developmental deformities and a great susceptibility to BCC (skin basal cell carcinoma) development. In mouse polymorphic variant found in ptc gene that confessed sensitivity to early postnatal squamous cell carcinoma (SCC) was identified with H-ras gene mutated (Wakabayashi et al. 2007). This also demonstrates that decrease in expression of ptc1 transgene promotes the formation of malignant SCCs.

8.3 Fas/Fas Ligand

Due to long-term exposure to UV rays, there occurs a change in expression of Fas receptor and ligand. Cell death can be activated by either of the two pathways:

intrinsic pathway and extrinsic pathway. Binding of death ligand to their receptor present on the damaged cell surface occurred in extrinsic pathway. Mutations occurring in Fas signaling, finally, leads to uncontrolled growth of cell and formation of tumor.

Increase in the expression of the FAS receptor on surfaces of melanoma cells inhibits tumor growth. However, FAS ligand has a high level of expression in tumors BCCs and SCCs; one of the reasons for this expression is that FAS ligand expression concedes the neoplasms to avoid harm occurring from the immune system. FAS receptor and ligand have an important role in the attainment of skin cancers and it is also found that polymorphisms, which are functional in the genes of Fas ligand and receptor, may be having association with cancer perceptivity. Two such polymorphisms detected in the promoter region of FAS gene are rs2234767 and rs1800682; other two in the FAS ligand gene (rs763110 in the promoter region and rs5030772 in the second intron) have been found to be the cause of cancer. Three out of four polymorphisms have functional effect (Sibley et al. 2003).

8.4 Ras Gene

The proto-oncogenes H-*ras*, K-*ras*, and N-*ras*en code 21-kDa proteins that share about 70% sequence homology. Ras proteins bind to GTP and intricate in cell signaling transduction pathway. Mutations occurred in the 12, 13, and 61 codons of gene ras and these codons are important for activation of ras gene and also for activation of all the other genes belonging to the *ras* family. According to reports mutations in *ras gene* occurred at a frequency rate of 10–40% in human skin carcinoma. However, patients suffering from xeroderma pigmentosum have a high rate of frequency (53%) to be occurred in ras gene; on the other hand, general population suffering from skin cancer has a frequency rate of 22% for mutations to be occurred in ras gene. Mutations in N-ras gene have been reported to occur in a mouse causing skin cancer due to UV rays (Daya-Grosjean et al. 1993).

It was the first oncogene discovered in which cellular transformation occurs due to point mutations. These mutations lead to senescence in cells, also effect the other functions which further cause alteration in the cell cycle and apoptosis control, and, finally, cause the induction of cellular transformation. The occurrence of mutations in RAS gene in cases of cutaneous squamous cell carcinomas or keratoacanthomas, a type of skin cancer due to UV rays, ranges between 3% and 30% as per the reports (Collado et al. 2005). Cancers develop after many years of exposure to chemotherapy, which causes genotoxic effects. Findings suggest that mutations in RAS genes occur more frequently due to abrasions, which arise mainly in skin damaged due to the sun.

8.5 Smoothened Protein

Smoothened (SMO) is a transmembrane protein, which is the major constituent of hedgehog (HH) and patched (*PTCH*) signaling network and is mainly involved in the cell growth regulation and also in development of embryos. The gene of this protein is a G protein-coupled receptor that has interaction with patched protein, which is a known receptor for hedgehog pathway proteins. Variations that affect the hedgehog and patched signaling pathway members have association with the growth of skin basal cell carcinomas (BCCs) (Li et al. 2011) due to out of control cell expression of transcription factors. Due to loss in functioning of ptc gene as per the UV rays, SMO signaling goes out of control, which leads to mutations and continuous activation of hedgehog pathway. Mutations in amino acid sequence of SMO that cause an unrestrained receptor activity are called activating mutations. These mutations in SMO protein contribute to about 6–21% of development of sporadic BCC (Xie et al. 1998).

9 Conclusion

Skin aging is largely influenced by both internal and external factors, inducing change at cellular and molecular level. These factors also influence rate of expression of the genes, which are associated with skin aging. The internal factor such as cellular senescence is associated with skin aging as it gets deposited with age in the case of skin and signifies aging. On the other hand, external agent such as UV contributes to skin aging by formation of ROS, cyclobutane pyrimidine dimers, and pyrimidine cross-linked dimers and, thus, leads to activation of p53 protein and apoptosis of keratinocytes expressed as suntan cells. The extended radiation exposure becomes more detrimental leading to repression of p53 protein and activation of mutation and photocarcinogenesis.

UV rays are the major cause of skin cancer. Mutations occurred due to long-term exposure to UV rays. These mutations occur in tumor suppressor genes and oncogenes and lead to DNA damage. So, damaged DNA needs to be repaired and mutations in genes involved in repair pathway are a major cause of skin cancer, as they further cause uncontrolled cell growth. Many genes and their proteins are involved in cell carcinomas. We have discussed the p53 gene as one of the major factors of skin cancer as mutation in p53 disrupts the DNA repair mechanism and transition mutations are responsible for loss in functioning of p53. Other genes and their proteins such as RAS (H-, K-, N-), Smoothened, Fas receptor/ligand, and patched1 are also known as causes of skin cancer due to exposure to UV rays. All these proteins are mostly membrane proteins affecting the signaling pathway of cell.

References

Bates, D., Maechler, M., & Dai, B. *The Ime4 package.*

Bernstein, E. F., Brown, D. B., Schwartz, M. D., Kaidbey, K., & Ksenzenko, S. M. (2004, February). The polyhydroxy acid gluconolactone protects against ultraviolet radiation in an in vitro model of cutaneous photoaging. *Dermatologic Surgery, 30*(2), 189–196.

Brash, D. E., Rudolph, J. A., Simon, J. A., Lin, A., McKenna, G. J., Baden, H. P., Halperin, A. J., & Ponten, J. (1991, November 15). A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proceedings of the National Academy of Sciences, 88*(22), 10124–10128.

Calles, C., Schneider, M., Macaluso, F., Benesova, T., Krutmann, J., & Schroeder, P. (2010, June 1). Infrared a radiation influences the skin fibroblast transcriptome: Mechanisms and consequences. *Journal of Investigative Dermatology, 130*(6), 1524–1536.

Chen, Y., & Struhl, G. (1996, November 1). Dual roles for patched in sequestering and transducing hedgehog. *Cell, 87*(3), 553–563.

Cho, S., Shin, M. H., Kim, Y. K., Seo, J. E., Lee, Y. M., Park, C. H., & Chung, J. H. (2009, August 1). Effects of infrared radiation and heat on human skin aging in vivo. *Journal of Investigative Dermatology Symposium Proceedings, 14*(1), 15–19.

Collado, M., Gil, J., Efeyan, A., Guerra, C., Schuhmacher, A. J., Barradas, M., Benguría, A., Zaballos, A., Flores, J. M., Barbacid, M., & Beach, D. (2005, August). Tumour biology: senescence in premalignant tumours. *Nature, 436*(7051), 642.

Daya-Grosjean, L., Robert, C., Drougard, C., Suarez, H., & Sarasin, A. (1993, April 1). High mutation frequency in ras genes of skin tumors isolated from DNA repair deficient xeroderma pigmentosum patients. *Cancer Research, 53*(7), 1625–1629.

DeBusk, F. L. (1972, April 1). The Hutchinson-Gilford progeria syndrome: Report of 4 cases and review of the literature. *The Journal of Pediatrics, 80*(4), 697–724.

Dumaz, N., Drougard, C., Sarasin, A., & Daya-Grosjean, L. (1993, November 15). Specific UV-induced mutation spectrum in the p53 gene of skin tumors from DNA-repair-deficient xeroderma pigmentosum patients. *Proceedings of the National Academy of Sciences, 90*(22), 10529–10533.

Dunn, J. A., McCance, D. R., Thorpe, S. R., Lyons, T. J., & Baynes, J. W. (1991, February 1). Age-dependent accumulation of N. epsilon.-(carboxymethyl) lysine and N. epsilon.-(carboxymethyl) hydroxylysine in human skin collagen. *Biochemistry, 30*(5), 1205–1210.

Dutta, A., Ruppert, J. M., Aster, J. C., & Winchester, E. (1993, September). Inhibition of DNA replication factor RPA by p53. *Nature, 365*(6441), 79.

Fisher, G. J., Kang, S., Varani, J., Bata-Csorgo, Z., Wan, Y., Datta, S., & Voorhees, J. J. (2002, November 1). Mechanisms of photoaging and chronological skin aging. *Archives of Dermatology, 138*(11), 1462–1470.

Fleming, T. H., Humpert, P. M., Nawroth, P. P., & Bierhaus, A. (2011). Reactive metabolites and AGE/RAGE-mediated cellular dysfunction affect the aging process–a mini-review. *Gerontology, 57*(5), 435–443.

Glass, D., Viñuela, A., Davies, M. N., Ramasamy, A., Parts, L., Knowles, D., Brown, A. A., Hedman, Å. K., Small, K. S., Buil, A., & Grundberg, E. (2013, July). Gene expression changes with age in skin, adipose tissue, blood and brain. *Genome Biology, 14*(7), R75.

Harman, D. (1956). Aging: A theory based on free radical and radiation chemistry. *Journal of Gerontology, 11*, 298–300.

Hutchinson, F. (1994, August 1). Induction of tandem-base change mutations. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 309*(1), 11–15.

Kolesnikov, N., Hastings, E., Keays, M., Melnichuk, O., Tang, Y. A., Williams, E., Dylag, M., Kurbatova, N., Brandizi, M., Burdett, T., & Megy, K. (2014, October 31). ArrayExpress update – Simplifying data submissions. *Nucleic Acids Research, 43*(D1), D1113–D1116.

Kollias, N., & Baqer, A. (1984, May). An experimental study of the changes in pigmentation in human skin in vivo with visible and near infrared light. *Photochemistry and Photobiology, 39*(5), 651–659.

Li, C., Chi, S., & Xie, J. (2011, August 1). Hedgehog signaling in skin cancers. *Cellular Signalling, 23*(8), 1235–1243.

Liebel, F., Kaur, S., Ruvolo, E., Kollias, N., & Southall, M. D. (2012, July 1). Irradiation of skin with visible light induces reactive oxygen species and matrix-degrading enzymes. *Journal of Investigative Dermatology, 132*(7), 1901–1907.

Maier, J. A., Statuto, M., & Ragnotti, G. (1993, September 1). Senescence stimulates U937-endothelial cell interactions. *Experimental Cell Research, 208*(1), 270–274.

Marchini, J., Howie, B., Myers, S., McVean, G., & Donnelly, P. (2007, July). A new multipoint method for genome-wide association studies by imputation of genotypes. *Nature Genetics, 39*(7), 906.

Maser, R. S., & DePinho, R. A. (2002, July 26). Connecting chromosomes, crisis, and cancer. *Science, 297*(5581), 565–569.

Ouhtit, A., Gorny, A., Muller, H. K., Hill, L. L., Owen-Schaub, L., & Ananthaswamy, H. N. (2000, December 1). Loss of Fas-ligand expression in mouse keratinocytes during UV carcinogenesis. *The American Journal of Pathology, 157*(6), 1975–1981.

Pinnell, S. R. (2003, January 1). Cutaneous photodamage, oxidative stress, and topical antioxidant protection. *Journal of the American Academy of Dermatology, 48*(1), 1–22.

Rosengardten, Y., McKenna, T., Grochová, D., & Eriksson, M. (2011 December). Stem cell depletion in Hutchinson–Gilford progeria syndrome. *Aging Cell, 10*(6), 1011–1020.

Schieke, S. M., Schroeder, P., & Krutmann, J. (2003, October). Cutaneous effects of infrared radiation: From clinical observations to molecular response mechanisms. *Photodermatology, Photoimmunology & Photomedicine, 19*(5), 228–234.

Sibley, K., Rollinson, S., Allan, J. M., Smith, A. G., Law, G. R., Roddam, P. L., Skibola, C. F., Smith, M. T., & Morgan, G. J. (2003, August 1). Functional FAS promoter polymorphisms are associated with increased risk of acute myeloid leukemia. *Cancer Research, 63*(15), 4327–4330.

Sklar, L. R., Almutawa, F., Lim, H. W., & Hamzavi, I. (2013). Effects of ultraviolet radiation, visible light, and infrared radiation on erythema and pigmentation: A review. *Photochemical & Photobiological Sciences, 12*(1), 54–64.

Stanulis-Praeger, B. (1987, March 1). Cellular senescence revisited: A review. *Mechanisms of Ageing and Development, 38*(1), 1–48.

Swisshelm, K., Ryan, K., Lee, X., Tsou, H. C., Peacocke, M., & Sager, R. (1994). Down-regulation of retinoic acid receptor beta in mammary carcinoma cell lines and its up-regulation in senescing normal mammary epithelial cells. *Cell Growth & Differentiation, 5*(2), 133–141.

Trautinger, F. (2001, October). Mechanisms of photodamage of the skin and its functional consequences for skin ageing. *Clinical and Experimental Dermatology, 26*(7), 573–577.

Valacchi, G., Fortino, V., & Bocci, V. (2005). The dual action of ozone on the skin. *The British Journal of Dermatology, 153*, 1096–1100.

Viña, J., Borrás, C., & Miquel, J. (2007). Theories of ageing. *IUBMB Life, 59*(4–5), 249–254.

Wakabayashi, Y., Mao, J. H., Brown, K., Girardi, M., & Balmain, A. (2007, February). Promotion of Hras-induced squamous carcinomas by a polymorphic variant of the patched gene in FVB mice. *Nature, 445*(7129), 761.

West, M. D., Pereira-Smith, O. M., & Smith, J. R. (1989, September 1). Replicative senescence of human skin fibroblasts correlates with a loss of regulation and overexpression of collagenase activity. *Experimental Cell Research, 184*(1), 138–147.

Xie, J., Murone, M., Luoh, S. M., Ryan, A., Gu, Q., Zhang, C., Bonifas, J. M., Lam, C. W., Hynes, M., Goddard, A., & Rosenthal, A. (1998, January). Activating smoothened mutations in sporadic basal-cell carcinoma. *Nature, 391*(6662), 90.

Zhu, P., Ren, M., Yang, C., Hu, Y. X., Ran, J. M., & Yan, L. (2012, February). Involvement of RAGE, MAPK and NF-κB pathways in AGEs-induced MMP-9 activation in HaCaT keratinocytes. *Experimental Dermatology, 21*(2), 123–129.

Ziegler, A., Jonason, A. S., Leffellt, D. J., Simon, J. A., Sharma, H. W., Kimmelman, J., Remington, L., Jacks, T., & Brash, D. E. (1994, December). Sunburn and p53 in the onset of skin cancer. *Nature, 372*(6508), 773.

Human Skin Stem Cells, Aging, and Possible Antiaging Strategies

Shambhoo Sharan Tripathi, Sandeep Singh, and Abhishek Kumar Singh

Abstract

Human skin is the largest organ of the body and it provides the first line of the defense system against environmental factors coming in contact by evading our ecosystem. Skin possesses notable regeneration capacity due to the presence of different types of stem cells including epithelial stem cells, melanocyte stem cells, mesenchymal stem-like cells, and progenitor cells. Moreover, the integrity of the skin is mainly maintained by epidermal stem cells. Skin and skin stem cells are more vulnerable toward aging process due to their direct contact with external stimuli including environmental pollutants, infection, and UV irradiation. Aging is a complex and multifactorial process mainly caused by imbalanced redox status, DNA mutation, and telomere shortening. The reactive oxygen species (ROS) overproduction is the major contributor of skin aging as ROS exert oxidative damage to macromolecules and cell organelles, which continuously accumulate and further accelerate aging process. Additionally, UV irradiation induces oxidative stress, overproduction of ROS, and DNA damage which collectively cause photoaging of the skin. This chapter summarizes the overall effects of oxidative stress on skin aging, and several antiaging strategies such as

Shambhoo Sharan Tripathi and Sandeep Singh have equally contributed to this chapter.

S. S. Tripathi
Bioscience and Bioengineering Department, Indian Institute of Technology-Bombay, Mumbai, India

Department of Biochemistry, University of Allahabad, Allahabad, India

S. Singh
Department of Biochemistry, University of Allahabad, Allahabad, India

A. K. Singh (✉)
Amity Institute of Neuropsychology and Neurosciences, Amity University Uttar Pradesh, Noida, India
e-mail: aksingh20@amity.edu

A. Dwivedi et al. (eds.), *Skin Aging & Cancer*,
https://doi.org/10.1007/978-981-13-2541-0_3

supplementation of nutritional antioxidants and autophagy modulation are also described to slow down the aging process of skin as well as skin diseases.

Keywords
Aging · Antioxidant · Oxidative stress · Photoaging · Skin · Stem cells

Skin is the complex and largest organ of the human body that offers protection against environmental stresses and serves as a sensory interface between the body and its surroundings. Human skin is made up of three main layers, viz., the epidermis, dermis, and subcutis. The cells of epidermal layer originate at the basal layer that persistently change their form as they rise to the surface of the skin and are ultimately shed; this is the natural cycle of epidermal layer. Anatomically, epidermal layer can be further divided into four layers, the basal layer, spinous layer, granular layer, and stratum corneum. All these layers made about 0.2-mm-thick, functioning barrier to protect the body from injurious external stimuli (Fisher et al. 1997, 2002; Kang et al. 2001). Skin formation is a continuous process in which new cells are formed at the basal layer and rise up to the stratum corneum. This pattern of cycle is very unique to each individual. Aging of individuals weakens skin metabolism; consequently injuries take a longer time to heal. The slower cyclic pattern of skin leads to the accumulation of dead skin cells on the surface resulting in thin-looking skin with a dull or rough appearance.

The dermal layer consists of hyaluronic acid, collagen, and elastin that are produced from fibroblast cells and help in maintaining skin hydration and firmness. However, this function can gradually decline as a result of skin aging, inflammation, and ultraviolet (UV) radiation exposure. The dermal layer provides firmness and elasticity to the skin. The epidermis is only 0.2 mm thick and comprises about 90% of skin's thickness. The dermis provides a greater influence on skin firmness and elasticity than the epidermis. The thickness of skin reduces with the progression of age due to loss of dermal collagen. Moreover, UV radiation exaggerates these aging-associated changes which results in coarse wrinkling, pigmentation (solar lentigines), and telangiectasia in UV-exposed areas.

1 Skin Stem Cells

The epidermis constantly self-renewed itself in order to resist the damage caused by their physical, chemical, and biological surroundings. This self-renewal ability of the epidermis is granted by adult stem cells and progenitor cells. Moreover, the dermis is divided into different lineages during embryonic development from morula to blastocyst stage of preimplantation. The first one is the trophectoderm and second is the inner cell mass (Adjaye et al. 2005; Gardner and Beddington 1988). The later one is a supplier of embryonic stem cells which possess self-renewal

capability and pluripotency (Thomson et al. 1998). Local stem cells play a significant role in the homeostatic maintenance of many organs and tissues. The best example is the epidermis, in which cellular turnover is high and also has high regenerative potential (Rando 2006). In the adult human body, there are different kinds of stem cells which involve in the renewal of the various cell types in the skin during normal homeostasis or wound repair. Normally, epidermal stem cells and melanoblasts are located in the skin.

Stem Cell Localization in Skin Different kinds of skin stem cells have been reported in heterogeneous environment of skin (Shi et al. 2006). The epidermal linings of skin are replaced several times in the course of a human lifetime. The undifferentiated stem cells in the basal layer divide continuously and produce differentiated descendants that eventually discarded with the time. Epidermal stem cells have two common features like other stem cells; first, they have self-renewal capacity for extended time periods, and, second, they can form multiple lineages after differentiation (Weissman et al. 2001). The stem cells possess specific cell markers such as p63, $\beta1^{high}$/melanoma chondroitin sulfate proteoglycan, and $a6^{high}$/$CD71^{dim}$ (Suzuki and Senoo 2012; Senoo et al. 2007; Pellegrini et al. 2001).

The multipotent stem cells that produced a series of differentiated cell types in the skin are to be found in a specific area that is adjoining with the epidermis, in the hair follicle, which is called as the bulge (Oshima et al. 2001; Christiano 2004; Morris et al. 2004). The follicle bulge region can develop into hair follicle epithelium, including hair shaft, inner root sheath, and outer root sheath. These cells have specified cellular markers named as Sox9, K15, Lgr5, CD34, Lhx2, NFIB, NFATC1, K15, PHLDA1, K19, CD200, etc. (Liu et al. 2003; Trempus et al. 2003; Jaks et al. 2008; Nowak et al. 2008; Sellheyer and Krahl 2011).

Epidermal Stem Cells Among a range of skin stem cells, epidermal stem cells are mainly linked to skin regeneration and tissue repair. Several studies suggested that epidermal stem cells are rare and occasionally dividing cells that produce short-lived and fast-dividing cells to accomplish the regeneration of the epidermis. They can be assumed as a key epidermal cell population conscientious for repairing skin injury. Although the majority of epidermal stem cells are resident of the basal layer in the epidermis, a few may also be present in the bulge region of the hair follicle as well as in the base of the sebaceous glands (Watt et al. 2006; Fuchs 2008). Epidermal stem cells, during their entire life cycle, are dispersed in two different cell types; one is the slow-growing cell type, in which epidermal stem cells are dormant. While in another fast-growing cell type, they are fast dividing and produce a large number of cells for the renewal of skin tissue. In the end, they go through several cell divisions prior to terminal differentiation to complete skin renewal. During skin damage, both epidermal stem cells and follicular stem cells play an important role in wound healing (Ito and Cotsarelis 2008; Ito et al. 2005; Taylor et al. 2000). At the time of injury, in the wounded region, hair follicle-derived epidermal stem cells along with pro-

genitor cells start to drift toward the wound spot. Thereafter, epidermal stem cells reactivate response to skin damage and assist to skin rejuvenation at the cellular level (Langton et al. 2008).

Melanocyte Stem Cells Melanocyte stem cells present in the skin are a supplier of transient fast-growing cells and differentiated melanocytes. There is little known about melanocyte stem cells and their function. The first time, a functional role (niche) for melanocyte stem cells has been reported in the bulge region of the hair follicle, also explained as the lower permanent position (Nishimura et al. 2002; Nishikawa and Osawa 2007). The stem cells are regulated by their microenvironment which includes other adjacent cells, secretary signaling proteins, and scaffold proteins of the extracellular matrix. The niche is very helpful for the population of the stem cell to evade the loss of the stem cell pools; in addition they are promoting dormancy so as to inhibit the overgrowth of cells. Stem cells as well as supportive cells within the niche may show overexpression of adhesion and extracellular matrix proteins including integrins or cadherins for the homeostasis of the cell (Raymond et al. 2009).

Mesenchymal Stem Cell-Like Cells and Neural Progenitor Cells Mesenchymal stem cell-like cells are present at the dermis, and after division, they form mesodermal parts and some cells of the neural system. They are CD34 negative and CD105, CD90, as well as CD70-specific positive surface markers (Garzón et al. 2013). Moreover, the follicle dermal papillae are the source of neural progenitor cells that can split into the glial and neural lineage, and like other organs or tissues, they shared similar cell markers such as nestin.

Hematopoietic Stem Cells These cells are situated at the follicle dermal papillae, and after differentiation, they form myeloid and erythroid cell lineages and express similar surface markers like in other organs or tissues.

Shortening of the Telomere, Loss of the Stem Cells, and Skin Aging Shortening of the telomere is a characteristic of aging which is manifested by the congregation of gene and DNA damage during cell divisions. On the basis of previous findings, it is clear that the nuclease, transferase, and polymerase enzyme activities of telomerase are accountable for the protection of telomeres against programmed cell death in response to DNA damage (Blasco 2005). However, the mechanisms behind the shortening of telomere in skin stem cell aging remain unknown (Counter et al. 2003; Friedrich et al. 2000; Nakamura et al. 2002). Skin contains adequate levels of telomerase enzyme and it is active only in few skin stem cells, basal epidermal cells (Bickenbach et al. 1998; Engelhardt et al. 1997; Taylor et al. 1996), the bulge component of the hair follicle, and keratinocytes (Boukamp 2005; Ramirez et al. 1997). Human epithelial cells or fibroblasts have low or sometimes no telomerase protein (Funk et al. 2000; Nakano et al. 1998; Zouboulis 2003). In dyskeratosis congenital patients, skin shows symptoms like early hair loss, hair graying, poor nail growth,

and skin atrophy which are related to shorting of telomeres that lead to defects in proliferation and function of skin stem cells and poor healing of the wound (Mason et al. 2005; Westin et al. 2007).

Consequently, germline and stem cells evolve mechanisms to prevent telomere abrasion and protect them from senescence. In this mechanism, telomerase gets activated, in which TERT, TERC, and dyskerin (DKC) are involved, and can extend the end of the chromosome with specific telomeric DNA sequences (Tomás-Loba et al. 2008; Collins and Mitchell 2002).

Estrogen, Stem Cells, and Skin Aging Estrogens have a multifunction role in skin aging; it can prevent the loss of collagen, augment the skin thickness, reinstate skin moisture, stop hair loss, as well as improve the wound healing (Brincat 2000; Azzi et al. 2005). Estrogen acts through the estrogen receptors (ERs) and can directly regulate fibroblast function. For collagen production, estrogen increases TGF production by fibroblasts (Ashcroft et al. 1997). Furthermore, estrogen can overturn epidermal atrophy by means of stimulation of the cell proliferation and synthesis of DNA in keratinocytes (Urano et al. 1995). Between menopause and the age of 60 years, the estrogen level 17-E2, DHEA, and progesterone rapidly fall and then exhibit a lower level plateau afterward (Morley 2001; Phillips et al. 2001).

It has been established that estrogen suppresses keratinocyte apoptosis. It also activates cell cycle checkpoint protein cyclin D2, predominantly by means of contact with a cell surface receptor GPR30, that further activates cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signaling pathway (Kanda and Watanabe 2004).

Estrogen has the ability to affect skin directly but its mechanism of action in regulation of skin progenitor cells is not very clear. On the other hand, at present, the role of estrogen in the regulation of different types of stem cells has already been made known. The alterations in hormone production with age play critical roles in the instigation of skin aging but the exact mechanisms involving skin stem cells as well as hormone deficiency remain unknown.

Reactive Oxygen Species (ROS), Stem Cells, and Skin Aging Excess of xanthine oxidase and nitric oxide synthase in tissues, under pathological stress conditions, produces heavy endogenous ROS. ROS production is drastically amplified after cigarette smoke, exposure to UV irradiation, and other abuse (Kohen 1999). It is very exciting that ROS mainly produced in mitochondria, as well as other cell organelles, play a crucial role in aging (Chance et al. 1979). In other studies, the exposure of UV irradiation to human skin fibroblasts increases oxidative stress and also increase the expression of signaling molecules p16INK4A and p53/p21WAF1/CIP1 that further cause the early senescence of fibroblasts and the conversion of the fibroblast into myofibroblast (Chen et al. 2008; Ruiter et al. 2002). It is well established that repeated exposure to solar UV irradiation is the principal environmental

factor for acceleration of skin aging process. In a study, skin samples were collected from sunlight exposure and sunlight-protected aged individuals and showed that the number of keratinocyte stem cells (KSCs) was considerably lower in photoaged than in the sun-protected skin (Kwon et al. 2008; Rass and Reichrath 2008).

UV Radiation and Photoaging of Skin Among the radiation UV radiation is one of natural mutagens liable for the leading proportion of environmentally induced skin diseases, including inflammation and erythema, skin cancer, and age-related changes (Elwood and Jopson 1997). The UV exposure leads to excessive ROS production such as superoxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen which are main contributors to the skin aging (Fig. 1). The effect of UV-induced oxidative stress on the skin aging is usually termed as photoaging. The UVA light mainly alters the physiology of dermis which is accountable for the progression of photoaging (Krutmann and Schroeder 2009). The UV-induced photoaging process also includes oxidative damage of DNA, especially the mtDNA (Berneburg et al. 1997). However, UVA and UVB affect only epidermal cells due to their limited penetration ability and thus contribute to the photoaging process (Krutmann and Schroeder 2009).

Thus, UV radiation affects the cell's macromolecules adversely through the production of ROS and other reactive free radicals (Meyskens et al. 2001). Increase in UV radiation in the environment might be an important factor for increasing incident of skin cancer and melanoma from last several years (Armstrong and Kricker 2001; Garbe and Leiter 2009). In childhood, UV protection is very important

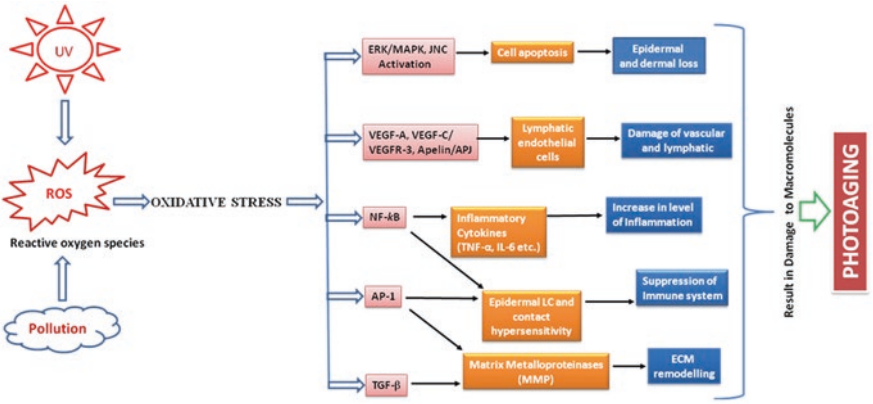

Fig. 1 Reactive oxygen species (ROS) produced from different sources initiate signaling pathways that contribute to change in skin structures and photoaging. The overproduction of ROS results in imbalanced redox status and oxidative stress which cause damage to various cellular macromolecules and eventually result in the development of photoaging of the skin. *Abbreviations*: *IL* interleukin, *TNF-a* tumor necrosis factor-a, *VEGF-A* vascular endothelial growth factor-A, *MAPK* multiple mitogen-activated protein kinase, *JNK* c-Jun N-terminal kinase, and *MMP* matrix metalloproteinases

because it induces deposition of DNA damage in adulthood and leads to skin cancer. There is the significant skin's anatomical difference between children and adults that makes easier for UV penetration and increases the risk of melanoma with excessive sun exposure before age of 10 years (Volkmer and Greinert 2011). Photoaging of skin is a combined effect of environmental and genetic factors and is mainly predisposed by exposure of UV radiation.

Skin stem cells are also susceptible to various genetic changes caused by harmful agents such as UV radiation; as a result tumor formation may occur (Perez-Losada and Balmain 2003). Interestingly, it has also been demonstrated that cyclobutane pyrimidine dimer (CPD) accumulates in epidermal basal cells upon chronic low-level UVB exposure. CPD-retaining basal cells (CRBCs) were also observed in human skin receiving sporadic sunlight exposure (Mitchell et al. 2001). Thus, CRBCs become targets for UV-induced skin cancer due to accumulation of DNA damage. On the other hand, the specific role of CRBCs in skin cancer induced by UV exposure has not yet been well documented.

2 Current Antiaging Strategies to Slow Down Skin Aging

During last couple of decades, a number of antiaging methods have been developed to slow down skin aging. The antiaging strategies include particularly preventive measurements, cosmetics, topical and systemic therapeutic agents, as well as invasive procedures. Since it is well documented that aging process is the consequent of overproduction of free radicals, nutritional supplements containing antioxidants are the best remedies for aging as they scavenge free radicals and defend the cells from oxidative injure. These nutritional antioxidants comprise of lipoic acid, coenzyme Q, carotenoids, vitamin C, and vitamin E and trace elements including selenium and copper (Fusco et al. 2007; Marini 2011; Berger 2005). There are two safeguards for the skin, endogenous antioxidant (enzymatic antioxidants and synthesis of melanin) and exogenous antioxidant (which we eat in the food). UV-induced photoaging is an outcome of the failure of endogenous anti-oxidative repair processes. After this incident, the skin becomes clinically explicit with age, and functional harm to the skin prevails. At the moment, it becomes necessary to consume supplementary antioxidants or to apply them on the skin in topical preparations (Poljsak et al. 2013).

Vitamin C is a water-soluble vitamin and presents in the skin predominantly that helps in protection of aqueous phase of the cells. Additionally, vitamin E protects the lipid portion of cell membranes and stratum corneum (Thiele 2001). The main concerns about the use of antioxidants include its compatibility, product stability, absorption inside the skin, and activation at the target spot. In the future, exact dosing and administration route will be more important for the use of an antioxidant to give more drug-like effect. UV-induced early photoaging, wrinkling, and pigmentary changes are measured as the most significant cutaneous manifestations. The antiaging strategies against UV-induced photoaging include the use of antioxidants, sunscreens to reduce UV exposure, and retinoids to promote collagen production.

Interestingly, combinatorial use of several strategies is the most powerful approach against UV-induced photoaging (Baumann 2007; Trautinger 2001).

Recently, the modulation of autophagy process has been shown to be an effective antiaging strategy against skin aging (Scherfer et al. 2013). Autophagy is a well-conserved cellular process accountable for the continuous removal of oxidatively damaged macromolecules and cell organelles (Singh et al. 2017). The keratinocytes with defective autophagy demonstrate augmented DNA damage, senescence, and anomalous change in lipid profile after oxidative stress (Song et al. 2017). Since autophagy has been demonstrated to regulate skin stem cells, slow down aging process, and deal with oxidative stress and microbial infection, the use of autophagy modulators provides a promising therapeutic strategy for alleviating skin aging and skin diseases (Li et al. 2016). Skin tissue engineering is another novel strategy which provides alternative regenerative medicinal approach for possible management of skin-related problems (Behera et al. 2017).

3 Conclusion

This chapter aims to give an overview of various types of stem cells found in the skin and their role in architecture and function of the skin. Moreover, the role of several factors including telomere shortening, UV irradiation, and oxidative stress in the general pathophysiology of the aging process of skin has also been critically reviewed. The process of aging in the skin is mainly driven by overproduction of reactive oxygen species which eventually lead to oxidative damage of macromolecules and cell organelles. Several antiaging strategies including nutritional antioxidant supplementation, autophagy modulation, and skin tissue engineering have also been discussed to slow down the process of aging in skin and skin diseases.

Acknowledgments S. S. Tripathi would like to acknowledge DSKPDF scheme of University Grants Commission, New Delhi, India, for providing financial support (F.4-2/2006(BSR)/BL/17-18/0381).

References

Adjaye, J., Huntriss, J., Herwig, R., et al. (2005). Primary differentiation in the human blastocyst: Comparative molecular portraits of inner cell mass and trophectoderm cells. *Stem Cells, 23*, 1514–1525. https://doi.org/10.1634/stemcells.2005-0113.

Armstrong, B. K., & Kricker, A. (2001). The epidemiology of UV induced skin cancer. *Journal of Photochemistry and Photobiology B, 63*, 8–18.

Ashcroft, G. S., Dodsworth, J., van Boxtel, E., et al. (1997). Estrogen accelerates cutaneous wound healing associated with an increase in TGF-beta1 levels. *Nature Medicine, 3*, 1209–1215.

Azzi, L., El-Alfy, M., Martel, C., & Labrie, F. (2005). Gender differences in mouse skin morphology and specific effects of sex steroids and dehydroepiandrosterone. *The Journal of Investigative Dermatology, 124*, 22–27. https://doi.org/10.1111/j.0022-202X.2004.23545.x.

Baumann, L. (2007). Skin ageing and its treatment. *The Journal of Pathology, 211*, 241–251. https://doi.org/10.1002/path.2098.

Behera, S. S., Das, U., Kumar, A., et al. (2017). Chitosan/TiO2composite membrane improves proliferation and survival of L929 fibroblast cells: Application in wound dressing and skin regeneration. *International Journal of Biological Macromolecules, 98*, 329–340. https://doi.org/10.1016/j.ijbiomac.2017.02.017.

Berger, M. M. (2005). Can oxidative damage be treated nutritionally? *Clinical Nutrition, 24*, 172–183. https://doi.org/10.1016/j.clnu.2004.10.003.

Berneburg, M., Gattermann, N., Stege, H., et al. (1997). Chronically ultraviolet-exposed human skin shows a higher mutation frequency of mitochondrial DNA as compared to unexposed skin and the hematopoietic system. *Photochemistry and Photobiology, 66*, 271–275.

Bickenbach, J. R., Vormwald-Dogan, V., Bachor, C., et al. (1998). Telomerase is not an epidermal stem cell marker and is downregulated by calcium. *The Journal of Investigative Dermatology, 111*, 1045–1052. https://doi.org/10.1046/j.1523-1747.1998.00420.x.

Blasco, M. A. (2005). Telomeres and human disease: Ageing, cancer and beyond. *Nature Reviews Genetics, 6*, 611–622. https://doi.org/10.1038/nrg1656.

Boukamp, P. (2005). Skin aging: A role for telomerase and telomere dynamics? *Current Molecular Medicine, 5*, 171–177.

Brincat, M. P. (2000). Hormone replacement therapy and the skin: Beneficial effects: The case in favor of it. *Acta Obstetricia et Gynecologica Scandinavica, 79*, 244–249.

Chance, B., Sies, H., & Boveris, A. (1979). Hydroperoxide metabolism in mammalian organs. *Physiological Reviews, 59*, 527–605. https://doi.org/10.1152/physrev.1979.59.3.527.

Chen, W., Kang, J., Xia, J., et al. (2008). p53-related apoptosis resistance and tumor suppression activity in UVB-induced premature senescent human skin fibroblasts. *International Journal of Molecular Medicine, 21*, 645–653.

Christiano, A. M. (2004). Epithelial stem cells: Stepping out of their niche. *Cell, 118*, 530–532. https://doi.org/10.1016/j.cell.2004.08.024.

Collins, K., & Mitchell, J. R. (2002). Telomerase in the human organism. *Oncogene, 21*, 564–579. https://doi.org/10.1038/sj.onc.1205083.

Counter, C. M., Press, W., & Compton, C. C. (2003). Telomere shortening in cultured autografts of patients with burns. *Lancet, 361*, 1345–1346. https://doi.org/10.1016/S0140-6736(03)13042-5.

Elwood, J. M., & Jopson, J. (1997). Melanoma and sun exposure: An overview of published studies. *International Journal of Cancer, 73*, 198–203.

Engelhardt, M., Kumar, R., Albanell, J., et al. (1997). Telomerase regulation, cell cycle, and telomere stability in primitive hematopoietic cells. *Blood, 90*, 182–193.

Fisher, G. J., Wang, Z. Q., Datta, S. C., et al. (1997). Pathophysiology of premature skin aging induced by ultraviolet light. *The New England Journal of Medicine, 337*, 1419–1428. https://doi.org/10.1056/NEJM199711133372003.

Fisher, G. J., Kang, S., Varani, J., et al. (2002). Mechanisms of photoaging and chronological skin aging. *Archives of Dermatology, 138*, 1462–1470.

Friedrich, U., Griese, E., Schwab, M., et al. (2000). Telomere length in different tissues of elderly patients. *Mechanisms of Ageing and Development, 119*, 89–99.

Fuchs, E. (2008). Skin stem cells: Rising to the surface. *The Journal of Cell Biology, 180*, 273–284. https://doi.org/10.1083/jcb.200708185.

Funk, W. D., Wang, C. K., Shelton, D. N., et al. (2000). Telomerase expression restores dermal integrity to in vitro-aged fibroblasts in a reconstituted skin model. *Experimental Cell Research, 258*, 270–278. https://doi.org/10.1006/excr.2000.4945.

Fusco, D., Colloca, G., Lo Monaco, M. R., & Cesari, M. (2007). Effects of antioxidant supplementation on the aging process. *Clinical Interventions in Aging, 2*, 377–387.

Garbe, C., & Leiter, U. (2009). Melanoma epidemiology and trends. *Clinics in Dermatology, 27*, 3–9. https://doi.org/10.1016/j.clindermatol.2008.09.001.

Gardner, R. L., & Beddington, R. S. (1988). Multi-lineage "stem" cells in the mammalian embryo. *Journal of Cell Science, 10*, 11–27.

Garzón, I., Miyake, J., González-Andrades, M., et al. (2013). Wharton's jelly stem cells: A novel cell source for oral mucosa and skin epithelia regeneration. *Stem Cells Translational Medicine, 2*, 625–632. https://doi.org/10.5966/sctm.2012-0157.

Ito, M., & Cotsarelis, G. (2008). Is the hair follicle necessary for normal wound healing? *The Journal of Investigative Dermatology, 128*, 1059–1061. https://doi.org/10.1038/jid.2008.86.

Ito, M., Liu, Y., Yang, Z., et al. (2005). Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nature Medicine, 11*, 1351–1354. https://doi.org/10.1038/nm1328.

Jaks, V., Barker, N., Kasper, M., et al. (2008). Lgr5 marks cycling, yet long-lived, hair follicle stem cells. *Nature Genetics, 40*, 1291–1299. https://doi.org/10.1038/ng.239.

Kanda, N., & Watanabe, S. (2004). 17beta-estradiol stimulates the growth of human keratinocytes by inducing cyclin D2 expression. *The Journal of Investigative Dermatology, 123*, 319–328. https://doi.org/10.1111/j.0022-202X.2004.12645.x.

Kang, S., Fisher, G. J., Voorhees, J. J. (2001). Photoaging: Pathogenesis, prevention, and treatment. *Clinics in Geriatric Medicine*, *17*, 643–659, v–vi.

Kohen, R. (1999). Skin antioxidants: Their role in aging and in oxidative stress–new approaches for their evaluation. *Biomedecine Pharmacotheraphy, 53*, 181–192. https://doi.org/10.1016/S0753-3322(99)80087-0.

Krutmann, J., & Schroeder, P. (2009). Role of mitochondria in photoaging of human skin: The defective powerhouse model. *The Journal of Investigative Dermatology. Symposium Proceedings, 14*, 44–49. https://doi.org/10.1038/jidsymp.2009.1.

Kwon, O. S., Yoo, H. G., Han, J. H., et al. (2008). Photoaging-associated changes in epidermal proliferative cell fractions in vivo. *Archives of Dermatological Research, 300*, 47–52. https://doi.org/10.1007/s00403-007-0812-3.

Langton, A. K., Herrick, S. E., & Headon, D. J. (2008). An extended epidermal response heals cutaneous wounds in the absence of a hair follicle stem cell contribution. *The Journal of Investigative Dermatology, 128*, 1311–1318. https://doi.org/10.1038/sj.jid.5701178.

Li, L., Chen, X., & Gu, H. (2016). The signaling involved in autophagy machinery in keratinocytes and therapeutic approaches for skin diseases. *Oncotarget, 7*, 50682–50697. https://doi.org/10.18632/oncotarget.9330.

Liu, Y., Lyle, S., Yang, Z., & Cotsarelis, G. (2003). Keratin 15 promoter targets putative epithelial stem cells in the hair follicle bulge. *The Journal of Investigative Dermatology, 121*, 963–968. https://doi.org/10.1046/j.1523-1747.2003.12600.x.

Marini, A. (2011). Beauty from the inside. Does it really work? *Hautarzt Z Dermatol Venerol Verwandte Geb, 62*, 614–617. https://doi.org/10.1007/s00105-011-2138-5.

Mason, P. J., Wilson, D. B., & Bessler, M. (2005). Dyskeratosis congenita – a disease of dysfunctional telomere maintenance. *Current Molecular Medicine, 5*, 159–170.

Meyskens, F. L., Farmer, P., & Fruehauf, J. P. (2001). Redox regulation in human melanocytes and melanoma. *Pigment Cell Research, 14*, 148–154.

Mitchell, D. L., Volkmer, B., Breitbart, E. W., et al. (2001). Identification of a non-dividing subpopulation of mouse and human epidermal cells exhibiting high levels of persistent ultraviolet photodamage. *The Journal of Investigative Dermatology, 117*, 590–595. https://doi.org/10.1046/j.0022-202x.2001.01442.x.

Morley, J. E. (2001). Androgens and aging. *Maturitas, 38*, 61–71. discussion 71–73.

Morris, R. J., Liu, Y., Marles, L., et al. (2004). Capturing and profiling adult hair follicle stem cells. *Nature Biotechnology, 22*, 411–417. https://doi.org/10.1038/nbt950.

Nakamura, K.-I., Izumiyama-Shimomura, N., Sawabe, M., et al. (2002). Comparative analysis of telomere lengths and erosion with age in human epidermis and lingual epithelium. *The Journal of Investigative Dermatology, 119*, 1014–1019. https://doi.org/10.1046/j.1523-1747.2002.19523.x.

Nakano, K., Watney, E., & McDougall, J. K. (1998). Telomerase activity and expression of telomerase RNA component and telomerase catalytic subunit gene in cervical cancer. *The American Journal of Pathology, 153*, 857–864. https://doi.org/10.1016/S0002-9440(10)65627-1.

Nishikawa, S.-I., & Osawa, M. (2007). Generating quiescent stem cells. *Pigment Cell Research, 20*, 263–270. https://doi.org/10.1111/j.1600-0749.2007.00388.x.

Nishimura, E. K., Jordan, S. A., Oshima, H., et al. (2002). Dominant role of the niche in melanocyte stem-cell fate determination. *Nature, 416*, 854–860. https://doi.org/10.1038/416854a.

Nowak, J. A., Polak, L., Pasolli, H. A., & Fuchs, E. (2008). Hair follicle stem cells are specified and function in early skin morphogenesis. *Cell Stem Cell, 3*, 33–43. https://doi.org/10.1016/j.stem.2008.05.009.

Oshima, H., Rochat, A., Kedzia, C., et al. (2001). Morphogenesis and renewal of hair follicles from adult multipotent stem cells. *Cell, 104*, 233–245.

Pellegrini, G., Dellambra, E., Golisano, O., et al. (2001). p63 identifies keratinocyte stem cells. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 3156–3161. https://doi.org/10.1073/pnas.061032098.

Perez-Losada, J., & Balmain, A. (2003). Stem-cell hierarchy in skin cancer. *Nature Reviews Cancer, 3*, 434–443. https://doi.org/10.1038/nrc1095.

Phillips, T. J., Demircay, Z., & Sahu, M. (2001). Hormonal effects on skin aging. *Clinics in Geriatric Medicine, 17*, 661–672. vi.

Poljsak, B., Dahmane, R., & Godic, A. (2013). Skin and antioxidants. *Journal of Cosmetic and Laser Therapy, 15*, 107–113. https://doi.org/10.3109/14764172.2012.758380.

Ramirez, R. D., Wright, W. E., Shay, J. W., & Taylor, R. S. (1997). Telomerase activity concentrates in the mitotically active segments of human hair follicles. *The Journal of Investigative Dermatology, 108*, 113–117.

Rando, T. A. (2006). Stem cells, ageing and the quest for immortality. *Nature, 441*, 1080–1086. https://doi.org/10.1038/nature04958.

Rass, K., & Reichrath, J. (2008). UV damage and DNA repair in malignant melanoma and nonmelanoma skin cancer. *Advances in Experimental Medicine and Biology, 624*, 162–178. https://doi.org/10.1007/978-0-387-77574-6_13.

Raymond, K., Deugnier, M.-A., Faraldo, M. M., & Glukhova, M. A. (2009). Adhesion within the stem cell niches. *Current Opinion in Cell Biology, 21*, 623–629. https://doi.org/10.1016/j.ceb.2009.05.004.

Ruiter, D., Bogenrieder, T., Elder, D., & Herlyn, M. (2002). Melanoma-stroma interactions: Structural and functional aspects. *The Lancet Oncology, 3*, 35–43.

Scherfer, C., Han, V. C., Wang, Y., et al. (2013). Autophagy drives epidermal deterioration in a Drosophila model of tissue aging. *Aging, 5*, 276–287. https://doi.org/10.18632/aging.100549.

Sellheyer, K., & Krahl, D. (2011). PHLDA1 (TDAG51) is a follicular stem cell marker and differentiates between morphoeic basal cell carcinoma and desmoplastic trichoepithelioma. *The British Journal of Dermatology, 164*, 141–147. https://doi.org/10.1111/j.1365-2133.2010.10045.x.

Senoo, M., Pinto, F., Crum, C. P., & McKeon, F. (2007). p63 is essential for the proliferative potential of stem cells in stratified epithelia. *Cell, 129*, 523–536. https://doi.org/10.1016/j.cell.2007.02.045.

Shi, C., Zhu, Y., Su, Y., & Cheng, T. (2006). Stem cells and their applications in skin-cell therapy. *Trends in Biotechnology, 24*, 48–52. https://doi.org/10.1016/j.tibtech.2005.11.003.

Singh, A. K., Kashyap, M. P., Tripathi, V. K., et al. (2017). Neuroprotection through Rapamycin-induced activation of autophagy and PI3K/Akt1/mTOR/CREB Signaling against amyloid-β-induced oxidative stress, synaptic/neurotransmission dysfunction, and Neurodegeneration in adult rats. *Molecular Neurobiology, 54*, 5815–5828. https://doi.org/10.1007/s12035-016-0129-3.

Song, X., Narzt, M. S., Nagelreiter, I. M., et al. (2017). Autophagy deficient keratinocytes display increased DNA damage, senescence and aberrant lipid composition after oxidative stress in vitro and in vivo. *Redox Biology, 11*, 219–230. https://doi.org/10.1016/j.redox.2016.12.015.

Suzuki, D., & Senoo, M. (2012). Increased p63 phosphorylation marks early transition of epidermal stem cells to progenitors. *The Journal of Investigative Dermatology, 132*, 2461–2464. https://doi.org/10.1038/jid.2012.165.

Taylor, R. S., Ramirez, R. D., Ogoshi, M., et al. (1996). Detection of telomerase activity in malignant and nonmalignant skin conditions. *The Journal of Investigative Dermatology, 106*, 759–765.

Taylor, G., Lehrer, M. S., Jensen, P. J., et al. (2000). Involvement of follicular stem cells in forming not only the follicle but also the epidermis. *Cell, 102*, 451–461.

Thiele, J. J. (2001). Oxidative targets in the stratum corneum. A new basis for antioxidative strategies. *Skin Pharmacology and Applied Skin Physiology, 14*(Suppl 1), 87–91. https://doi.org/10.1159/000056395.

Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., et al. (1998). Embryonic stem cell lines derived from human blastocysts. *Science, 282*, 1145–1147.

Tomás-Loba, A., Flores, I., Fernández-Marcos, P. J., et al. (2008). Telomerase reverse transcriptase delays aging in cancer-resistant mice. *Cell, 135*, 609–622. https://doi.org/10.1016/j.cell.2008.09.034.

Trautinger, F. (2001). Mechanisms of photodamage of the skin and its functional consequences for skin ageing. *Clinical and Experimental Dermatology, 26*, 573–577.

Trempus, C. S., Morris, R. J., Bortner, C. D., et al. (2003). Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. *The Journal of Investigative Dermatology, 120*, 501–511. https://doi.org/10.1046/j.1523-1747.2003.12088.x.

Urano, R., Sakabe, K., Seiki, K., & Ohkido, M. (1995). Female sex hormone stimulates cultured human keratinocyte proliferation and its RNA- and protein-synthetic activities. *Journal of Dermatological Science, 9*, 176–184.

Volkmer, B., & Greinert, R. (2011). UV and children's skin. *Progress in Biophysics and Molecular Biology, 107*, 386–388. https://doi.org/10.1016/j.pbiomolbio.2011.08.011.

Watt, F. M., Lo Celso, C., & Silva-Vargas, V. (2006). Epidermal stem cells: An update. *Current Opinion in Genetics & Development, 16*, 518–524. https://doi.org/10.1016/j.gde.2006.08.006.

Weissman, I. L., Anderson, D. J., & Gage, F. (2001). Stem and progenitor cells: Origins, phenotypes, lineage commitments, and transdifferentiations. *Annual Review of Cell and Developmental Biology, 17*, 387–403. https://doi.org/10.1146/annurev.cellbio.17.1.387.

Westin, E. R., Chavez, E., Lee, K. M., et al. (2007). Telomere restoration and extension of proliferative lifespan in dyskeratosis congenita fibroblasts. *Aging Cell, 6*, 383–394. https://doi.org/10.1111/j.1474-9726.2007.00288.x.

Zouboulis, C. C. (2003). Intrinsic skin aging. A critical appraisal of the role of hormones. *Hautarzt Z Dermatol Venerol Verwandte Geb, 54*, 825–832. https://doi.org/10.1007/s00105-003-0581-7.

UVR-Induced Skin Cancer

Jyoti Singh

Abstract

From the epidemiological point of view, it is suggested that regular contacts to UVR irradiation since our childhood are the primary cause of skin tumors. UVR-induced ROS production caused DNA damage, immune suppression, and deactivation of tumor suppression genes or overactivation of proto-oncogene. These processes are interconnected with each other. Cyclobutane pyrimidine dimers (CPDs) and (6–4) photoproducts are main key products of DNA damage. Our body system has DNA repair mechanism which mainly involves nuclear excision repair and base excision repair pathways. Defect in repair pathways and continuous accumulation of mutation lead to photocarcinogenesis. DNA lesions are an important molecular mediator in initiation of immunosuppression which has a important role in the induction of UVR-mediated skin cancer. DNA damage induced by UVR involves inhabitation of cell cycle progress or apoptosis. P53 plays an important role in cell cycle; it arrests the G1 phage and removes DNA lesion. Mutations in P53 gene come into light as an early event in the progress of UV-induced skin cancers.

Keywords

Epidemiological · Photocarcinogenesis · DNA damage · Mutation

J. Singh (✉)
Photobiology laboratory, Systems Toxicology and Health Risk Assessment Group, CSIR-Indian Institute of Toxicology Research, Lucknow, India

Academy of Scientific and Innovative Research, New Delhi, India

A. Dwivedi et al. (eds.), *Skin Aging & Cancer*,
https://doi.org/10.1007/978-981-13-2541-0_4

1 Introduction

Constant exposures to solar radiation since our childhood epidemiologically appeared to be the primary cause of skin tumors. Ultraviolet B (UVB) radiation has a minor portion of sunlight to which people get exposed. It caused DNA damage such as cyclobutane pyrimidine dimers (CPDs) and (6–4) photoproducts provoke change in skin cells and promote the of skin cancer development. Additionally UVB is known for the upregulation of gene expression which involves various types of skin-associated disease via the involvement of different types of intracellular signal transduction pathway. UVB has a significant impact for the induction and development of nonmelanoma skin cancer and basal and squamous cell carcinomas and may also promote other skin disorders such as sunburn, photoaging, and actinic keratosis. UVB exposure also causes a huge amount of reactive oxygen species generation in skin cells, causing oxidative stress and DNA damage in skin cells. Our skin has a well-developed antioxidant defense system for the protection from oxidative stress. Ultraviolet radiation are proved to induced DNA damage directly via the formation of DNA adduct and indirectly through oxidative stress.

2 Role of ROS in UVR-Induced Skin Cancer

Both nuclear and mitochondrial DNA are damaged by ROS and UVR. According to recent study it is found that skin aging is associated with mtDNA damage and mutations. It is indicated that mtDNA mutation is a sensitive biomarker for ROS-mediated damage and UVR exposure (Birch Machin et al. 2013). In addition to this previously it has been demonstrated that ROS-mediated DNA damage and defects in DNA repair hOGG1 gene could accounted for photocarcinogenesis were associated with patients suffering from BCC (Chaisiriwong et al. 2016). In in vitro and In vivo models genomic damage that results from UVB and UVA exposure preferentially induces C-T transitions. UVB directly affect the when its photon absorbed by DNA molecule, which leads to the scuffling in nucleotide arrangement and cause defect in DNA structure. Cyclobutane pyrimidine dimers (CPDs) and (6–4) photoproducts are the major outcome of this UVB-induced rearrangement of nucleotides. UVB absorption leads to the formation of CPDs formed in adjacent pyrimidine nucleotides by the cycloaddition between C5 and C6. While 6–4 PPs photoproduct is result of covalent bond formation between two adjacent pyrimidine bases generates, which further changes in to its Dewar valence isomer (Rochette et al. 2003). Meanwhile our cell system provokes DNA repair mechanism and halts cell division to restrict further DNA damage. In prokaryote, photolyase enzyme is responsible for removal of UV-induced DNA damage where enzyme binds at CPDs and 6–4 photoproduct formation site split it and back it to undamaged site. However human beings do not have this type of repair system. Humans have the nucleotide excision repair (NER) pathway in response to UVR-induced DNA damage. There are total nine major proteins that work as NER in mammalian cells. RAD23s, RPA, ERCC1 proteins, and others also contribute in nucleotide excision repair (de Laat et al.

1999). NER-defective syndrome like xeroderma pigmentosum (XP) noticeably discloses the importance of neuclotide excision repair mechanism on human body system. The augmented UVR induced skin aging photoaging and tremendous high frequency of skin tumorigenesis of XP patients suggest UVR-induced DNA damage could be the cause of serious damage to the human skin (Schuch et al. 2017). NER future divided into two sub pathways, which initiation mechanism is different from each other but once they recognised the DNA damage after damage recognition, both pathways activates the same molecular mechanism to remove damage. These two pathways are described as transcription-coupled NER (TC-NER) and global genome NER (GG-NER). TC-NER as its name suggested eliminates damage from transcribed strand of active genes. This mechanism of repair does not affect transcription machinery. While GG-NER removes damage part from the entire genome, it has been observed that this process is very slow and inefficient because this includes scanning of the whole genome for a single DNA damage. In general DNA repair pathways classified into 4 steps: (i) Reorganization of DNA lesion (ii) DNA unwinding, (iii) double incision and flanking the DNA lesion site, after that removes damaged fragment, and (iv) DNA repair and religation.

Since last two decades there has been an explosive clinical and experimental research for reactive oxygen species (ROS), producing from the metabolism of molecular oxygen and includes singlet oxygen ($^1O_2$), superoxide anion radical ($O_2-.$) and the highly reactive hydroxyl radical ($\cdot OH$). The existence of ROS in aerobic cells occurs with the balance of various type of biochemical antioxidants. ROS is tightly regulated and very important for signaling pathway but when the homeostasis between ROS generation and antioxidant disturbed, it react with lipid and other biomolecule and induced harmful effect (Sharma et al. 2012).

3 Role of Immune System in UVR-Induced Skin Cancer

From the last few years it has been observed that it's UVB radiation has ability to suppress the human immune defence system. UVR can inhibit immune reponse locally and systematicaly, as the amount of UVR exposure increased (de Gruijl 2008). UVR action mechanism is diffenernt in comparison to immunosuppressive drugs which involve in suppress of the immune system in a general fashion while UVR works in antigen-specific fashion. UVR mainly behaves due the generation of T cells which play major role in immune supression T cells shows therapeutic nature in the cure of (auto) immune-mediated diseases. UVR-induced immunosuppression plays a important role in development of UVR-mediated skin cancer, which is one of the most frequently occurring cancers worldwide (Hanneman et al. 2006). Therefore, study of the molecular pathways behind this gives the better understanding of UVR innduced skin cancer and illustrates different strategies to more effectively detect and diagnosed of photocarcinogensis cases. Additionally, T cells (Treg) characterization (phenotypes and function) gives a brief idea of the mechanism of their activation by the UVR which will further help to T cells model in the cure and diagonsis of skin associated cancers. Model to study

immunosuppression induced by UVR is contact hypersensitivity (CHS) induced by UVR. If contact allergens directly applied on skin it resulted in antigen-specific sensitization which observed by swelling of ear after few days later upon the application of the same allergen. If these potent contact allergens applied on skin which has already exposed to UVR, incident of contact hypersensitivity is suppressed and prevented (Toews et al. 1980). Characterized UV-induced Treg (UVR-Treg) express well CD4 and CD25, CTLA-4, GITR, neuropilin and interleukin (IL)-10 (Schwarz et al. 2010). Thus, they appear to represent a certain subtype of CD4 + CD25+ Treg. DNA damage caused by UVR reported as the main event to trigger UVR-induced immunosuppression (Kripke et al. 1992). Damage Induced by UVR are cyclobutane pyrimidine dimers and 6–4 photoproducts. Exogenous DNA repair enzymes promote removal of DNA lesions which further reduce UVR-induced immunosuppression, proving that DNA lesions is a essential molecular mediator in initiation of immunosuppression (Kripke et al. 1992). IL-12 was the first cytokine which is able to prevent UVR-induced immunosuppression (Müeller et al. 1995).It has been elucidated that Intraperitoneal injection of IL-12 Inhibits the induction of CHS. Similarly, IL-12 treatment also stops Treg production in UVR-exposed mice. IL-12 showed tendency to reduce UVR-induced DNA damage (Schwarz et al. 2002) IL-12 decreased apoptotic cell death in both in vitro and in vivo cell model which caused by UVR DNA damage (Kulms et al. 1999). IL-12 activates nucleotide excision repair (NER), endogenous DNA repair system which was confirmed by this study where IL-12 on UVR-induced cell death was not observed in Xpa knock-out mice which are deficient in NER (Schwarz et al. 2002).

4 Tumor Suppressor Genes and Oncogene in UVR-Induced Skin Cancer

Skin cancer caused by UVR often involves the deactivation of tumor suppression genes or overactivation of proto-oncogene. Tumor suppressor gene and gene product of p53 is the most diverse and complex molecules play an important role in different cellular functions. p53 gene protects our skin cells from DNA-damage caused by chronic UVB exposure. DNA lesions induced by UVR activates different molecular mechanisms to remove or repair DNA dmage, halts the cell cycle progression, or inhibits the apoptosis by transcriptional activation of p53-related genes, such as Bax, MDM2 and p21(Benjamin and Ananthaswamy 2007). In general, p53 occurs in very low amount but in response to UVR damage amount of this gene increased, it arrests the G1 phage where DNA lesions are removed before the mitosis phages. As a result, Consequently, p53 helps in repair of DNA dmage or removal of cells which have huge amount of damaged DNA (Levine 1997). The mutations in p53 gene observed as an early genetic changes in the progress of UV-induced skin cancers. Normal-appearing sun-exposed skin has shown thousands of p53-mutant cell clones. Fortunately, our skin system is equipped with complex antioxidant enzymes systems that constantly observe and detect most of the damage caused by UV light. On the other hand, any mistake or fault in DNA repair mechanshim and replication can lead

to mutations in the genome. Chronic exposure to sunlight induced several mutations in key genes which accumulated in high amount in skin and induced skin cancer. Seeing as UVR-induced associated cancer does not arise suddenly after UVR exposure mutated ras or p53 genes have to remain dormant for prolonged times (Setlow 1982). It has been found that only after UV irradiation CC to TT mutations in the p53 gene were detected in cultured human skin cells, and formation CC to TT dimer is increased at higher dose of UVR exposure. Two methods such as mutant allele-specific PCRs and ligase chain reactions are used to detect presumptive UV-specific p53 gene mutations in UV-exposed normal skin. CC– > TT mutations involving codons 247 and 248 of the p53 gene are associated with an increased risk of BCC (Nakazawa et al. 1994).

5 Conclusion

UVR is main cause of skin cancer; it promotes skin cells to become cancerous cells. Too much exposure to sunlight (UVR) or other sources such as sunbeds can damage the genetic material. International Agency for Research on Cancer (IARC) classifies UV radiation as "carcinogenic to humans." It is recommended that seek the shade, especially between 10 AM and 4 PM. If you are going outside apply sunscreen lotion to your entire body before 30 min.

References

Benjamin, C. L., & Ananthaswamy, H. N. (2007). p53 and the pathogenesis of skin cancer. *Toxicology and Applied Pharmacology, 224*(3), 241–248.

Birch-Machin, M. A., Russell, E. V., & Latimer, J. A. (2013). Mitochondrial DNA damage as a biomarker for ultraviolet radiation exposure and oxidative stress. *British Journal of Dermatology, 169*, 9–14.

Chaisiriwong, L., Wanitphakdeedecha, R., Sitthinamsuwan, P., Sampattavanich, S., Chatsiricharoenkul, S., Manuskiatti, W., & Panich, U. (2016). A case-control study of involvement of oxidative DNA damage and alteration of antioxidant defense system in patients with basal cell carcinoma: Modulation by tumor removal. *Oxidative Medicine and Cellular Longevity, 2016*, 5934024.

De Gruijl, F. R. (2008). UV-induced immunosuppression in the balance. *Photochemistry and Photobiology, 84*(1), 2–9.

de Laat, W. L., Jaspers, N. G., & Hoeijmakers, J. H. (1999). Molecular mechanism of nucleotide excision repair. *Genes & Development, 13*(7), 768–785.

Hanneman, K. K., Cooper, K. D., & Baron, E. D. (2006). Ultraviolet immunosuppression: mechanisms and consequences. *Dermatologic Clinics, 24*(1), 19–25.

Kripke, M. L., Cox, P. A., Alas, L. G., & Yarosh, D. B. (1992). Pyrimidine dimers in DNA initiate systemic immunosuppression in UV-irradiated mice. *Proceedings of the National Academy of Sciences, 89*(16), 7516–7520.

Kulms, D., Pöppelmann, B., Yarosh, D., Luger, T. A., Krutmann, J., & Schwarz, T. (1999). Nuclear and cell membrane effects contribute independently to the induction of apoptosis in human cells exposed to UVB radiation. *Proceedings of the National Academy of Sciences, 96*(14), 7974–7979.

Levine, A. J. (1997). p53, the cellular gatekeeper for growth and division. *Cell, 88*(3), 323–331.

Mueller, G., Saloga, J., Germann, T., Schuler, G., Knop, J., & Enk, A. H. (1995). IL-12 as mediator and adjuvant for the induction of contact sensitivity in vivo. *The Journal of Immunology, 155*(10), 4661–4668.

Nakazawa, H., English, D., Randell, P. L., Nakazawa, K., Martel, N., Armstrong, B. K., & Yamasaki, H. (1994). UV and skin cancer: Specific p53 gene mutation in normal skin as a biologically relevant exposure measurement. *Proceedings of the National Academy of Sciences, 91*(1), 360–364.

Rochette, P. J., Therrien, J. P., Drouin, R., Perdiz, D., Bastien, N., Drobetsky, E. A., & Sage, E. (2003). UVA-induced cyclobutane pyrimidine dimers form predominantly at thymine–thymine dipyrimidines and correlate with the mutation spectrum in rodent cells. *Nucleic Acids Research, 31*(11), 2786–2794.

Schuch, A. P., Moreno, N. C., Schuch, N. J., Menck, C. F. M., & Garcia, C. C. M. (2017). Sunlight damage to cellular DNA: Focus on oxidatively generated lesions. *Free Radical Biology and Medicine, 107*, 110–124.

Schwarz, A., Ständer, S., Berneburg, M., Böhm, M., Kulms, D., van Steeg, H., Grosse-Heitmeyer, K., Krutmann, J., & Schwarz, T. (2002). Interleukin-12 suppresses ultraviolet radiation-induced apoptosis by inducing DNA repair. *Nature Cell Biology, 4*(1), 26.

Schwarz, A., Noordegraaf, M., Maeda, A., Torii, K., Clausen, B. E., & Schwarz, T. (2010). Langerhans cells are required for UVR-induced immunosuppression. *Journal of Investigative Dermatology, 130*(5), 1419–1427.

Setlow, R. B. (1982). DNA repair, aging, and cancer. *National Cancer Institute Monograph, 60*, 249–255.

Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany, 2012*.

Toews, G. B., Bergstresser, P. R., & Streilein, J. W. (1980). Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *The Journal of Immunology, 124*(1), 445–453.

Ultraviolet Radiation-Induced Immunomodulation: Skin Ageing and Cancer

Garima Suman and Shankar Suman

Abstract

The prolonged exposure to ultraviolet radiation causes serious health issues to human beings. Skin is the largest and most visible organ of our body and most exposed to UV radiation. UV radiation is one of the most important environmental stressors responsible for causing skin damage. In this chapter, we have given an overview of the chronic effects of ultraviolet irradiation such as photoageing and cancer. The mechanisms underlying these effects include free radical-induced cell injury, direct and indirect DNA damage, genetic mutations, etc. We have elaborated on the risk factors and causes of UV-induced skin ageing and cancer in details. We have also enlisted the preventive measures that can minimize the UV-mediated chronic pathologies including premature ageing and cancer.

Keywords

UV-R · Photoageing · Skin cancer · DNA damage

1 Introduction

Ultraviolet radiation (UV) is one of the principal components of solar radiation. It has a shorter wavelength than visible light and is invisible to human eyes. It can be divided into UVA, UVB and UVC based on the wavelength typically lying between 100–400 nm, of which UVA has the longest wavelength at 320–400 nm, UVB

G. Suman
Department of Radio-Diagnosis, Tata Memorial Hospital, Mumbai, India

S. Suman (✉)
Department of Microbiology & Immunology, School of Medicine, Meharry Medical College, School of Medicine, Nashville, TN, USA

A. Dwivedi et al. (eds.), *Skin Aging & Cancer*,
https://doi.org/10.1007/978-981-13-2541-0_5

intermediate between 290–320 nm and the shortest is UVC ranging between 290–100 nm. UVA and UVB are prevalent in the sun rays reaching us, because UVC is mostly filtered out by ozone layers and unreachable to the earth surface. In particular, UVA accounts for the highest amount of radiations on the earth crust.

The level of UV radiation varies throughout the day depending on different geographical locations; thereby the impact of UV rays is also varied in different populations in different geographical areas. Studies have shown that the various factors pertaining to a particular geographical area, including altitude, latitude and surrounding environment determine the UV strength because the atmospheric particles like dust and vapour scatter UV photons. It is understood that the higher the altitude, the higher is the exposure of UV rays because of the lesser atmospheric shield. Studies have revealed that people living in the high-altitude areas are highly prone to develop skin cancers and the risk increases by 2% with every 10 m rise in altitude (Haluza et al. 2014). Additionally, the risk of getting skin cancer is also higher in people working at high altitudes such as mountain guides, pilots, etc., (Hammar et al. 2002, Lichte et al. 2010). Also, UV ray is stronger near the equator due to sunlight directly hitting the earth. That is why melanoma is more prevalent in Caucasians living near the equator (Flament et al. 2013). A study by Cicarma et al. showed that melanoma risk is increased by 2–2.5-fold with every 10° decrease in latitude (Cicarma et al. 2010). Another study revealed that the risk is increased 1.5 times in latitudes 0–20° (Chang et al. 2009).

More than 80% of UV rays are reflected back from snow especially in high-altitude areas and hence the exposure to humans in those areas is doubled. Apart from geographical locations, lifestyle is also a major factor that predisposes to deleterious effects of UV rays. For example, the risk of melanoma is higher in the urban population than the people living in the rural regions. It can be possibly due to certain soical factors like increasing trends of artificial tanning and easy access to tanning booths in urban areas as compared to rural areas.

Skin being completely exposed to the outside environment is highly prone to be affected by environmental factors. Skin is highly sensitive to Ultraviolet radiations and its prolonged exposure causes several short-term and long-term effects on skin pigmentations (Coelho et al. 2009). People with fair skin are more sensitive to UV rays than people with darker skin (Hu et al. 2004). The exposure to UV rays is a major causative factor in melanomagenesis (Gilchrest and Eller 1999) and photoageing. The details of UV radiations and their harmful effects on the skin have been elaborated in the subsequent sections.

2 Effects of UV Radiation Exposure

The UV radiations penetrate the skin layers causing inevitable changes in the cells which includes cellular deregulation and genetic mutation. Throughout our lifetime, UV radiation accumulates several damages on the skin cells, thereby leading to skin cancer and ageing. The UV radiations damage DNA which leads to initiation of cellular senescence and carcinogenesis (Amaro-Ortiz et al. 2014). Prolonged exposure

is thought to be a major predisposing factor; however, the immunomodulation due to the exposure varies among the individuals and geographical regions as well.

Human skins have an adaptive tanning response to solar radiation. The UV exposure favours cellular damage response which induces melanin production by melanocytes. The deposited melanin in the keratinocytes increases pigmentations in the skin which can protect from the adverse effect of the UV radiations to the skin. A study also revealed that our body can protect the UV insults on skins by activating cutaneous melanocortin 1 receptor (MC1R) signalling response pathways (Amaro-Ortiz et al. 2014; Suzuki et al. 1996).

Ultraviolet B damages the skins more than UVA and causes acute effects such as sunburn and chronic effects like skin cancer and photoageing. The carcinogenic potential of UVB is well reported by the World Health Organization (WHO) as well as US Department of Health and Human Services. The UV radiation is mainly considered to cause non-melanoma skin cancers such as basal cell carcinoma and squamous cell carcinoma. Although UV rays have been strongly implicated in the process of melanomagenesis, the incidence of melanoma is far less than non-melanoma skin cancers.

The UVA rays are 30–50 times more prevalent in sun rays, due to equal relative intensity during all daylight hours. It can also penetrate through the clouds and glasses. Also, due to its longer wavelength, UVA radiation penetrates deeper into the skin than UVB. It is well known that UVA causes skin ageing and wrinkling. For a long time, it was believed that UVA caused no significant damage to the epidermis of the skin which is the primary site of most skin cancers.

However, it is now well established with the studies over the past two decades that UVA damages the keratinocytes in the basal layer of the epidermis and contributes to the development of skin cancer.

UVA is the dominant ray emitted from sunlamps used in tanning salons. The high-pressure sunlamps used in tanning salons emit 12 times higher doses of UVA as compared to that of sunlight. Excessive UVA exposure causes injury to the skin's DNA. Skin responds to this damage by causing proliferation of the basal layer of the epidermis, which includes melanocytes. Melanocyte proliferation causes skin darkening or tan. This cumulative DNA damage resulting from UVA can lead to skin cancer.

People who are regular users of artificial tanning booths are 2.5 times more likely to develop squamous cell carcinoma and 1.5 times more likely to develop basal cell carcinoma. According to research, melanoma risk is increased by 75% after the first exposure to tanning sunlamp, particularly in young people.

UVA phototherapy has shown in beneficial role in a number of dermatological disorders (Zandi et al. 2012). Understanding the molecular mechanism behind skin disorders is still an ongoing process; however it has been revealed that UVA induces collagenase or matrix metalloproteinase-1 expression level as well as can deplete immune cells like T cells, Langerhans and mast cells in the dermis. Furthermore, studies have also revealed that UVA exposure can stimulate the endothelial for forming new blood vessels. UVA phototherapy has a therapeutic role in a number of

dermatologic disorders like psoriasis, vitiligo, dermatitis, scleroderma, acne vulgaris and cutaneous T cell lymphoma, etc.

3 Immunomodulation by UV Radiation

The overexposure to UV radiation may suppress proper functioning of the body's immune system and the skin's natural defences. For example, the skin normally mounts a defence against foreign invaders such as microbial infections. However, overexposure to UV radiation can weaken the immune system to reduce the protection against these invaders. Furthermore, solar UV exposure is another major factor associated with age-related changes (Makrantonaki and Zouboulis 2007).

Ultraviolet radiation elicits immunomodulation by inhibiting antigen presentation and altering cytokine levels. Although it does not show a direct role in immunosuppression, several pieces of literature give evidence of UV immunosuppression through antigen-specific fashion. Ultraviolet-exposed skin induces antigen-specific tolerance, which damages the sensitization on the skins to mediate immunosuppression. The UVB induces DNA damage as a major molecular target for immunosuppression. Regulatory T cells also known as immunosuppression responsive cells are suppressed by UV rays. UV rays also damage the Langerhans cells in the skin. Interleukin-12 exhibits the capacity to reduce DNA damage to prevent UV-based immunosuppression and tolerance. Recently, studies have given more emphasis on understanding the molecular mechanism underlying UV-induced immunosuppression (Gonzalez Maglio et al. 2016). The immunosuppression can deregulate the various stress response pathways in the skin cell. The skin homeostasis is maintained by mesenchymal stem cells and epidermal stem cells. The reduction of their number impairs homeostasis and causes skin ageing and cancer. These stem cells maintain self-renewable and multipotent ability to repair the damage by stressors; however, UV rays contribute to a reduction in both kinds of the cells augmenting the ageing process (Panich et al. 2016).

The high UVB exposure interferes with normal immunological functions and enhances infection rate. UVB radiation also initiates systemic immunosuppression and delayed-type hypersensitivity response. A study has also shown that even the use of sunscreen does not prevent UV-induced immunological suppression. Moreover, the UVB susceptibility on the immune systems of individuals with different skin types and color has been well discussed by Vermeer et al. (1991). Tropical populations are subjected to high UVB flux and a very high effective dose within a few hours of exposure to midday sun. The weakening of immunological response also dramatically decreases the survival rate of the populations.

4 Ultraviolet Radiation and Ageing

Exposure to UV rays attributes to nearly 80% of visible cutaneous signs of ageing, such as skin wrinkles, freckles, loss of skin tone and rough and dry skin (Grant 2008). These signs are seen on the sun-exposed parts of the body like the face, neck

and dorsal surface of limbs. Overexposure to UV rays causes the premature appearance of these signs also called as photoageing and predisposes the individual to actinic keratosis and melanoma. UV radiation, particularly UVB, is considered mutagenic which can cause cellular damage by both direct and indirect mechanisms (Cleaver and Crowley 2002; Krutmann et al. 2012; Wei et al. 2003), defective cellular signalling (Prunier et al. 2012) and photoageing (Stohs Sidney 1995). The study revealed that these changes in the skin increase the susceptibility to melanoma incidence (Wendt et al. 2012). Photoageing and skin cancer risk correlates with cumulative duration of UV exposure. The UV exposure and DNA modulation begin early with sun exposure starting at a young age and hence it is encouraged to protect overexposure since the paediatric years. The correlation of age and sun exposure study showed that the excessive exposure before 10 years of age significantly increases the risk. The study revealed that UV contributed to the structural and anatomical difference in skin enabled to penetrate to UV rays and increased risk of adult melanoma (Volkmer and Greinert 2011). It is also estimated that 80% of lifetime UV exposure occurs before the age of 20 (Amaro-Ortiz et al. 2014).

UV radiations caused several ill effects on human cells to initiate premature ageing through a variety of mechanisms including:

1. Oxidative stress

Free radicals are produced during normal metabolic activities like mitochondrion oxidative phosphorylation and peroxide metabolism. The imbalance in the production of free radicals over the scavenging power can increase the oxidative stress in the cells. The levels of reactive oxygen species (ROS) increase with UV exposure. It can alter the cellular cytoskeleton by damaging lipids, proteins and nucleic acids (Zastrow et al. 2009; Meyskens Jr. et al. 2001). The DNA modifications can also be a result of oxidative injury, for example, 7,8-dihydro-8-oxoguanine (8-oxoguanine; 8-OH-dG) (Schulz et al. 2000). Solar radiation can increase the level of oxidative stress on the skin, which causes the loss of skin elasticity (Langton et al. 2010; Naylor et al. 2011). Moreover, oxidative stress and ROS deregulates signalling mechanisms in the cell to interfere with the genome and transcriptional activities in the cells, which can contribute to photoageing, loosening of skin elasticity, etc. Among the most overt sign of UV exposure is wrinkling in the skin. Wrinkling is majorly due to insufficient activity of antioxidant enzymes against UV-induced oxidative stress. Increase in ROS level can deplete the glutathione and trigger antioxidant enzymes such as catalase and SOD. Catalase acts on the excess hydrogen peroxide molecules to convert into water and molecular oxygen and UV radiations reduces the activity of catalase enzyme (Song et al. 2009). Melanocortin 1 receptor (MC1R) and cAMP signalling are implicated as antioxidant regulator and a study has shown that pharmacological activation of MC1R/cAMP pathways can lower the level of UVA induced ROS via NADH oxidase and cAMP signalling (Henri et al. 2012). A study evidently showed that M1CR activation on melanocytes produce menlanocyte stimulating hormone (MSH) for melanin production to reduce oxidative stress upon UV exposure (Kadekaro et al. 2012).

Human skin contains various antioxidants, which protect the skin from deleterious effects of free radicals generated by various environment stressors. These antioxidants include endogenous enzymatic antioxidants such as GSH peroxidase (GPx), SOD and catalase. Apart from it there are various nonenzymatic low-molecular-weight antioxidants present in the skin such as vitamin E isoforms, vitamin C, GSH, uric acid and ubiquinol are decreased in dermis and epidermis by UV radiations (Shindo et al. 1993). Similarly, manganese superoxide dismutase (MnSOD) is a key mitochondrial enzyme that disposes superoxide generated during the respiratory chain reaction. The superoxide anion breaks into hydrogen peroxide (H2O2), which is further detoxified by GSH peroxidase into water and molecular oxygen. The specific mechanism as to how each of these antioxidants protects from UV ray-induced free radicals is not clear. However, it is known that overexposure to UV rays depletes the naturally occurring antioxidants in the skin (McArdle et al. 2002). It is well known that not all skin cells are equally exposed to the same level of oxidative stress. Keratinocytes in the epidermis are the most susceptible cells. It has been shown that UV light causes more damage to the antioxidant defence system in the epidermis than the dermis of the skin. A study by Sander et al. (2002) has shown that the damage to cellular biomolecules caused by acute exposure to UV rays can be prevented by prior antioxidant treatment.

2. Cellular senescence and shortening of telomere

Cellular senescence reduces the cellular capacity to divide and proliferate, which is sometimes in conjunction with shortening of telomeres. One of the reasons for the cellular senescence is oxidative stress, which is thought to directly lead to photoageing (Liu et al. 2012; Sakura et al. 2013; Velarde et al. 2012; Yun et al. 2011). Yokoo et al. found that exposing cells to a pro-oxidant agent (H2O2) impaired telomerase function, which eventually resulted in telomere shortening, decreased proliferation and cellular enlargement (Yokoo et al. 2004). It also triggered cellular damage pathways leading to initiation of photoageing.

3. Degradation of dermal mechanical barriers

Prolonged exposure to the radiation causes damage to skin layers which act as a mechanical barrier. The outermost layer of the epidermis, stratum corneum, is known as the first line of defence which is damaged by the exposure. The severe macroscopic damages by alteration by UV rays include chapping and cracking, which lead to infection, scarring and abnormal desquamation. Cohesion and mechanical strength of skin cells are associated with intercellular lipids and desmosomes. The exposure can markedly decrease the strength of intercellular bridges by increasing biochemical alterations in them. These changes lead to decrease in the natural ability of resistance thus compromising critical skin barriers (Biniek et al. 2012). The study also revealed that large doses of UV radiations may inactivate carotenoids and promote degradation of dermal collagen and elastin in the skin.

5 UV Exposure and Skin Cancer

Ultraviolet radiation is considered to be a major etiologic factor in the development of skin cancers all around the world; however, people with fair skin are more predisposed. The major cause for UV-induced skin cancer is through its immunosuppressive effects.

UV radiations in sunlight and artificial sources like tanning devices and therapeutic devices pose a considerable risk in the development of skin cancer (Narayanan et al. 2010). UV radiation is the major cause of skin neoplasm in occupationally exposed people including farmers, workers involved in the constructions and some public service workers too (Young 2009). Ultraviolet radiation causes the highest percentage of environmentally induced skin pathologies and skin cancer is one of the major consequences. The radiation causes direct and indirect damage which result in mutagenesis in skin cells. Shorter UV rays are vulnerable to modify DNA covalent pyrimidine bonds, which leads to breaks and change in the nucleotide structure. This DNA lesion distorts the DNA structure and accumulates DNA mutation. The skin cell may accumulate 100,000 such lesions in a day. As we discussed in the above paragraphs, UV radiation damages macromolecules and produces free radicals. Cyclobutane pyrimidine dimer containing 5-methylcytosine is a mutation induced by sunlight in mammalian cells. Studies have showed that these photoproducts are more responsible for UVB-induced mutations. The most common UVB mutagenesis includes deamination of cytosine and 5-methylcytosine within cyclobutane pyrimidine dimer. McAuliffe and Blank showed that UVA can alter the protein structures of the stratum corneum as it is assumed that structural changes in the proteins take place with UV exposure (McAuliffe and Blank 1991). The molecular mechanism of UV-induced cancer is still not well understood. However, several research studies have showed that UV radiation can activate p38 mitogen-activated protein kinase (MAPK), Jun N-terminal kinase (JNK) and nuclear factor-κB (NF-κB)-associated pathways in skin cells (Muthusamy and Piva 2013).

The major types of skin cancer reported due to UV exposure are broadly categorized into melanoma and non-melanoma cancers (Sarasin 1999). Melanoma is one of the most disastrous skin cancers which accounts for more than 75% skin cancer-related deaths worldwide. It has a propensity to rapidly metastasize to distant body parts. Childhood overexposure to the sun is a major risk and genetic factor and immunomodulation are other factors that accelerate the disease progression. Another skin cancer type is non-melanoma skin cancers, which are less deadly than melanomas. However, if this cancer is untreated it can be equally serious as melanomas. Two major types of non-melanoma skin cancers are basal cell carcinoma and squamous cell carcinomas. Basal cell carcinoma typically appears like a small nodule with rolled edges and grows slowly. It may penetrate the underlying structures; however only rarely does it show distant metastasis. On the other hand, SCC appears as red and scaly patches or a large mass and spreads rapidly to other parts of the body.

6 Prevention Strategies Against UV-Mediated Damage

6.1 Dietary Antioxidants

Natural antioxidants in fruits and vegetables are believed to influence the skin's anti-oxidative defence and might have a role in protection from UV rays as suggested by a few laboratory studies done on animal models. The dietary nutrients that promote healthy skin, includes vitamins A, C and E, certain fatty acids and many other plant-derived ingredients. These have shown beneficial effects on skin to protect from other dermatological disorders. For example, vitamin C can induce procollagen activity, decrease pigmentation problems and inflammatory conditions against UVA and UVB rays (Poljsak and Dahmane 2012). Biochemically, vitamin C also regenerates vitamin E from its chromanoxyl radical (Packer et al. 1979). Selenium and niacin are required for glutathione activity. Studies have shown that routine use of green tea or its extract whether in topical or oral form is protective against UV rays. Green tea is obtained from the fresh leaves of the plant *Camellia sinensis*. Green tea is rich in polyphenols and catechins. Polyphenols are one of the rich sources of anti-inflammatory, anticarcinogenic and anti-ageing potentials. Nowadays there is an increasing trend of combining different antioxidants, taking exogneous antioxidant orally and appying on skin can be a synergistic prevention strategy against oxidative stress and DNA damge occurred by UV radiations (Poljsak and Dahmane 2012). The effectiveness of green tea improves when taken along with vitamin C.

7 Cosmeceutical Products

Topical retinoids remain one of the top agents for treating photoageing. Their application not only repairs the photoaged skin but also prevents photoageing (Serri and Iorizzo 2008). Retinoids promote collagen I synthesis (Griffiths et al. 1993), reorganization of packed collagen fibres (Yamamoto et al. 1995) and an increase in number of type VII anchoring fibrils. However, topical therapy with retinoids often results in unwanted cutaneous dermatitis, including erythema and scaling (Mukherjee et al. 2006; Weiss et al. 1988).

8 Sunscreen

Sunscreen is a topically applied cream, which is formulated to protect or to treat the area from sunburn. It aids our body defence systems to absorb or reflect or scatter sunrays and its ability to do so is determined by sun protection factor (SPF). SPF of a sunscreen compares the amount of time required to produce sunburn on sunscreen-protected skin to the same amount of time needed to cause sunburn on an unprotected skin. For example, SPF 15 will take 15 times longer time to redden the skin than an unprotected skin. Currently, there are 17 ingredients approved by the FDA

to use as sunscreens, which can be broadly categorized as physical and chemical sunscreens. Physical sunscreens contain some insoluble particles that reflect the harmful rays. Chemical filter forms a thin, protective film on the surface of the skin to penetrate through absorbing UV rays. Nowadays most of the sunscreens are a mixture of chemical and physical ingredients. Some examples of these ingredients are avobenzone, ecamsule, oxybenzone, titanium dioxide and zinc oxide.

9 Natural Sunscreen

Aloe vera gel, widely used in cosmetics, blocks both UVA and UVB rays and can act as the sunscreen. Tomatoes are rich in lycopene, a powerful carotenoid antioxidant. Lycopene may reduce the damaging effect of UV light on the skin. It can also boost the protection against both the short-term and cumulative effects of sun exposure (Stahl et al. 2006). Other naturally occurring products like peach, pomegranate, almonds and grapes are also rich in polyphenols and have sunscreen action (Kim et al. 2002; Weerakkody et al. 2010). Forskolin is a plant *Plectranthus barbatus* (Coleus forskohlii) having a pharmacologically active compound which protects from UV rays (Amaro-Ortiz et al. 2014).

10 Physical Protection

While our body's natural defences, dietary components and cosmeceuticals do help in photoprotection, still additional measures are essential for protection from UV rays. These measures include sun avoidance and physical protection. Sun avoidance is the limiting exposure during the peak UV hours (10 am–4 pm), avoiding UV-reflective surfaces such as sand, snow and water. On the other hand, physical protection means preventing the exposure by wearing photoprotective dresses (broad-brimmed hat and long sleeves) or blocking UV rays using filters such as UV-blocking films on windows.

11 Summary

The solar UV radiation is ubiquitous for human life in the production of vitamin D; however, it also causes several ill effects if overexposed. These ill effects include DNA damage, photoageing and malignancy. Photoageing and skin cancer have been well studied so far. UV radiation is well known to penetrate into the skin to initiate photochemical reactions, which leads to detrimental effects. The acute effects of UV irradiation are erythema, pain and swelling. However, chronic exposure leads to photoageing. Both common UV radiations (UVA and UVB) show adverse effects like premature skin ageing, eye damage (including cataracts) and skin cancers. The excessive UV radiation causes genetic mutations as well as damages DNA that can be a key reason to develop skin cancer. UV radiation induces

chemical changes in the cells in which it is absorbed. UV radiations also suppress the immune system, thereby reducing our defence system to cope with skin-associated problems. For minimizing the mal-effects associated with UV radiations, prevention strategies should be emphasized for every individual to prevent UV overexposure and resulting pathologies.

References

Amaro-Ortiz, A., Yan, B., & D'Orazio, J. A. (2014). Ultraviolet radiation, ageing and the skin: Prevention of damage by topical cAMP manipulation. *Molecules (Basel, Switzerland), 19*, 6202–6219.

Biniek, K., Levi, K., & Dauskardt, R. H. (2012). Solar UV radiation reduces the barrier function of human skin. *Proceedings of the National Academy of Sciences, 109*, 17111.

Chang, Y.-m., Barrett, J. H., Bishop, D. T., Armstrong, B. K., Bataille, V., Bergman, W., et al. (2009). Sun exposure and melanoma risk at different latitudes: A pooled analysis of 5700 cases and 7216 controls. *International Journal of Epidemiology, 38*, 814–830.

Cicarma, E., Juzeniene, A., Porojnicu, A. C., Bruland, Ø. S., & Moan, J. (2010). Latitude gradient for melanoma incidence by anatomic site and gender in Norway 1966–2007. *Journal of Photochemistry and Photobiology B: Biology, 101*, 174–178.

Cleaver, J. E., & Crowley, E. (2002). UV damage, DNA repair and skin carcinogenesis. *Frontiers in Bioscience, 7*, d1024–d1043.

Coelho, S. G., Choi, W., Brenner, M., Miyamura, Y., Yamaguchi, Y., Wolber, R., et al. (2009). Short- and long-term effects of UV radiation on the pigmentation of human skin. *The Journal of Investigative Dermatology Symposium Proceedings, 14*, 32–35.

Flament, F., Bazin, R., Laquieze, S., Rubert, V., Simonpietri, E., & Piot, B. (2013). Effect of the sun on visible clinical signs of ageing in Caucasian skin. *Clinical, Cosmetic and Investigational Dermatology, 6*, 221–232.

Gilchrest, B. A., & Eller, M. S. (1999). DNA photodamage stimulates melanogenesis and other photoprotective responses. *The Journal of Investigative Dermatology Symposium Proceedings, 4*, 35–40.

Gonzalez Maglio, D. H., Paz, M. L., & Leoni, J. (2016). Sunlight effects on immune system: Is there something Else in addition to UV-induced immunosuppression? *BioMed Research International, 2016*, 1934518.

Grant, W. B. (2008). The effect of solar UVB doses and vitamin D production, skin cancer action spectra, and smoking in explaining links between skin cancers and solid tumours. *European Journal of Cancer, 44*, 12–15.

Griffiths, C., Russman, A. N., Majmudar, G., Singer, R. S., Hamilton, T. A., & Voorhees, J. J. (1993). Restoration of collagen formation in photodamaged human skin by Tretinoin (retinoic acid). *New England Journal of Medicine, 329*, 530–535.

Haluza, D., Simic, S., & Moshammer, H. (2014). Temporal and spatial melanoma trends in Austria: An ecological study. *International Journal of Environmental Research and Public Health, 11*, 734–748.

Hammar, N., Linnersjo, A., Alfredsson, L., Dammstrom, B. G., Johansson, M., & Eliasch, H. (2002). Cancer incidence in airline and military pilots in Sweden 1961-1996. *Aviation, Space, and Environmental Medicine, 73*, 2–7.

Henri, P., Beaumel, S., Guezennec, A., Poumes, C., Stoebner, P. E., Stasia, M. J., et al. (2012). MC1R expression in HaCaT keratinocytes inhibits UVA-induced ROS production via NADPH oxidase- and cAMP-dependent mechanisms. *Journal of Cellular Physiology, 227*, 2578–2585.

Hu, S., Ma, F., Collado-Mesa, F., & Kirsner, R. S. (2004). UV radiation, latitude, and melanoma in US Hispanics and blacks. *Archives of Dermatology, 140*, 819–824.

Kadekaro, A. L., Chen, J., Yang, J., Chen, S., Jameson, J., Swope, V. B., et al. (2012). Alpha-melanocyte-stimulating hormone suppresses oxidative stress through a p53-mediated signalling pathway in human melanocytes. *Molecular Cancer Research, 10*, 778–786.

Kim, Y. H., Yang, H. E., Park, B. K., Heo, M. Y., Jo, B. K., & Kim, H. P. (2002). The extract of the flowers of Prunus persica, a new cosmetic ingredient, protects against solar ultraviolet-induced skin damage in vivo. *Journal of Cosmetic Science, 53*, 27–34.

Krutmann, J., Morita, A., & Chung, J. H. (2012). Sun exposure: What molecular photodermatology tells us about its good and bad sides. *Journal of Investigative Dermatology, 132*, 976–984.

Langton, A. K., Sherratt, M. J., Griffiths, C. E. M., & Watson, R. E. B. (2010). Review article: A new wrinkle on old skin: The role of elastic fibres in skin ageing. *International Journal of Cosmetic Science, 32*, 330–339.

Lichte, V., Dennenmoser, B., Dietz, K., Häfner, H. M., Schlagenhauff, B., Garbe, C., et al. (2010). Professional risk for skin cancer development in male mountain guides – A cross-sectional study. *Journal of the European Academy of Dermatology and Venereology, 24*, 797–804.

Liu, L., Xie, H., Chen, X., Shi, W., Xiao, X., Lei, D., et al. (2012). Differential response of normal human epidermal keratinocytes and HaCaT cells to hydrogen peroxide-induced oxidative stress. *Clinical and Experimental Dermatology, 37*, 772–780.

Makrantonaki, E., & Zouboulis, C. C. (2007). Characteristics and Pathomechanisms of endogenously aged skin. *Dermatology, 214*, 352–360.

McArdle, F., Rhodes, L. E., Parslew, R., Jack, C. I. A., Friedmann, P. S., & Jackson, M. J. (2002). UVR-induced oxidative stress in human skin in vivo: Effects of oral vitamin C supplementation. *Free Radical Biology and Medicine, 33*, 1355–1362.

McAuliffe, D. J., & Blank, I. H. (1991). Effects of UVA (320-400 nm) on the barrier characteristics of the skin. *The Journal of Investigative Dermatology, 96*, 758–762.

Meyskens, F. L., Jr., Farmer, P., & Fruehauf, J. P. (2001). Redox regulation in human melanocytes and melanoma. *Pigment Cell Research, 14*, 148–154.

Mukherjee, S., Date, A., Patravale, V., Korting, H. C., Roeder, A., & Weindl, G. (2006). Retinoids in the treatment of skin ageing: An overview of clinical efficacy and safety. *Clinical Interventions in Aging, 1*, 327–348.

Muthusamy, V., & Piva, T. J. (2013). A comparative study of UV-induced cell signalling pathways in human keratinocyte-derived cell lines. *Archives of Dermatological Research, 305*, 817–833.

Narayanan, D. L., Saladi, R. N., & Fox, J. L. (2010). Review: Ultraviolet radiation and skin cancer. *International Journal of Dermatology, 49*, 978–986.

Naylor, E. C., Watson, R. E. B., & Sherratt, M. J. (2011). Molecular aspects of skin ageing. *Maturitas, 69*, 249–256.

Packer, J. E., Slater, T. F., & Willson, R. L. (1979). Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature, 278*, 737.

Panich, U., Sittithumcharee, G., Rathviboon, N., & Jirawatnotai, S. (2016). Ultraviolet radiation-induced skin aging: The role of DNA damage and oxidative stress in epidermal stem cell damage mediated skin aging. *Stem Cells International, 2016*, 14.

Poljsak, B., & Dahmane, R. (2012). Free radicals and extrinsic skin aging. *Dermatology Research and Practice, 2012*, 135206.

Prunier, C., Masson-Genteuil, G., Ugolin, N., Sarrazy, F., & Sauvaigo, S. (2012). Aging and photoageing DNA repair phenotype of skin cells – Evidence toward an effect of chronic sun exposure. *Mutation Research, 736*, 48–55.

Sakura, M., Chiba, Y., Kamiya, E., Furukawa, A., Kawamura, N., Niwa, M., et al. (2013). Spontaneous occurrence of photo ageing-like phenotypes in the dorsal skin of old SAMP1 mice, an oxidative stress model. *Experimental Dermatology, 22*, 62–64.

Sander, C. S., Chang, H., Salzmann, S., Müller, C. S. L., Ekanayake-Mudiyanselage, S., Elsner, P., et al. (2002). Photoaging is associated with protein oxidation in human skin in vivo. *Journal of Investigative Dermatology, 118*, 618–625.

Sarasin, A. (1999). The molecular pathways of ultraviolet-induced carcinogenesis. *Mutation Research, 428*, 5–10.

Schulz, I., Mahler, H. C., Boiteux, S., & Epe, B. (2000). Oxidative DNA base damage induced by singlet oxygen and photosensitization: Recognition by repair endonucleases and mutagenicity. *Mutation Research, 461*, 145–156.

Serri, R., & Iorizzo, M. (2008). Cosmeceuticals: Focus on topical retinoids in photoaging. *Clinics in Dermatology, 26*, 633–635.

Shindo, Y., Witt, E., & Packer, L. (1993). Antioxidant defense mechanisms in murine epidermis and dermis and their responses to ultraviolet light. *Journal of Investigative Dermatology, 100*, 260–265.

Song, X., Mosby, N., Yang, J., Xu, A., Abdel-Malek, Z., & Kadekaro, A. L. (2009). Alpha-MSH activates immediate defence responses to UV-induced oxidative stress in human melanocytes. *Pigment Cell & Melanoma Research, 22*, 809–818.

Stahl, W., Heinrich, U., Aust, O., Tronnier, H., & Sies, H. (2006). Lycopene-rich products and dietary photoprotection. *Photochemical & Photobiological Sciences, 5*, 238–242.

Stohs Sidney, J. (1995). The role of free radicals in toxicity and disease. *Journal of Basic and Clinical Physiology and Pharmacology, 6*(3–4), 205–228.

Suzuki, I., Cone, R. D., Im, S., Nordlund, J., & Abdel-Malek, Z. A. (1996). Binding of melanotropic hormones to the melanocortin receptor MC1R on human melanocytes stimulates proliferation and melanogenesis. *Endocrinology, 137*, 1627–1633.

Velarde, M. C., Flynn, J. M., Day, N. U., Melov, S., & Campisi, J. (2012). Mitochondrial oxidative stress caused by Sod2 deficiency promotes cellular senescence and ageing phenotypes in the skin. *Ageing, 4*, 3–12.

Vermeer, M., Schmieder, G. J., Yoshikawa, T., van den Berg, J. W., Metzman, M. S., Taylor, J. R., & Streilein, J. W. (1991). Effects of ultraviolet B light on cutaneous immune responses of humans with deeply pigmented skin. *Journal of Investigative Dermatology, 97*, 729–734.

Volkmer, B., & Greinert, R. (2011). UV and children's skin. *Progress in Biophysics and Molecular Biology, 107*, 386–388.

Weerakkody, P., Jobling, J., Infante, M. M. V., & Rogers, G. (2010). The effect of maturity, sunburn and the application of sunscreens on the internal and external qualities of pomegranate fruit grown in Australia. *Scientia Horticulturae, 124*, 57–61.

Wei, Q., Lee, J. E., Gershenwald, J. E., Ross, M. I., Mansfield, P. F., Strom, S. S., et al. (2003). Repair of UV light-induced DNA damage and risk of cutaneous malignant melanoma. *Journal of the National Cancer Institute, 95*, 308–315.

Weiss, J. S., Ellis, C. N., Headington, J. T., & Voorhees, J. J. (1988). Topical tretinoin in the treatment of ageing skin. *Journal of the American Academy of Dermatology, 19*, 169–175.

Wendt, J., Schanab, O., Binder, M., Pehamberger, H., & Okamoto, I. (2012). Site-dependent actinic skin damage as risk factor for melanoma in a central European population. *Pigment Cell & Melanoma Research, 25*, 234–242.

Yamamoto, O., Bhawan, J., Solares, G., Tsay, A. W., & Gilchrest, B. A. (1995). Ultrastructural effects of topical tretinoin on dermo-epidermal junction and papillary dermis in photodamaged skin. A controlled study. *Experimental Dermatology, 4*, 146–154.

Yokoo, S., Furumoto, K., Hiyama, E., & Miwa, N. (2004). Slow-down of age-dependent telomere shortening is executed in human skin keratinocytes by hormesis-like-effects of trace hydrogen peroxide or by anti-oxidative effects of pro-vitamin C in common concurrently with reduction of intracellular oxidative stress. *Journal of Cellular Biochemistry, 93*, 588–597.

Young, C. (2009). Solar ultraviolet radiation and skin cancer. *Occupational Medicine (London), 59*, 82–88.

Yun, J. S., Pahk, J. W., Lee, J. S., Shin, W. C., Lee, S. Y., & Hong, E. K. (2011). Inonotus obliquus protects against oxidative stress-induced apoptosis and premature senescence. *Molecules and Cells, 31*, 423–429.

Zandi, S., Kalia, S., & Lui, H. (2012). UVA1 phototherapy: A concise and practical review. *Skin Therapy Letter, 17*, 1–4.

Zastrow, L., Groth, N., Klein, F., Kockott, D., Lademann, J., Renneberg, R., et al. (2009). The missing link – Light-induced (280–1,600 nm) free radical formation in human skin. *Skin Pharmacology and Physiology, 22*, 31–44.

UVR and Role of Pigmentation in Skin Aging and Cancer

Neeraj Agarwal

Abstract

This chapter is focused on understanding the role of ultraviolet radiation (UVR) and skin pigmentation in aging and cancer. We all are exposed to solar radiation reaching the earth surface. Solar radiation reaching the earth surface is mainly comprised of visible and ultraviolet radiation. Excessive exposure to ultraviolet rays can be detrimental and lead to more severe diseases like cancer. Skin pigmentation plays a major role in controlling the effect of radiation exposure. People exposed to solar radiation are differentially affected based on the amount of pigmentation present in their skin. Melanin pigment present in the skin gives the color of skin; i.e., more melanin means darker skin and also absorbs the UVR preventing from its harmful effects.

Keywords

Erythema · Electromagnetic radiation · Sunburn · Melanoma

1 Introduction

1.1 Solar and UV Radiation

Sunlight is the ultimate source of thermodynamically usable energy for the metabolic processes of all the living system. All the living organisms inhabiting the earth's surface have to cope with sun's radiation. No doubt, sunlight keeps the earth warm and with no sunlight, our planet earth will be very cold and lifeless. Light is

N. Agarwal (✉)
Department of Medicine, Division of Hematology/Oncology, Samuel Oschin Comprehensive Cancer Institute Cedars-Sinai Medical Center, Los Angeles, CA, USA
e-mail: neeraj.agarwal@cshs.org

A. Dwivedi et al. (eds.), *Skin Aging & Cancer*,
https://doi.org/10.1007/978-981-13-2541-0_6

an indispensable component of all the life forms from the beginning of life. About 350 years ago, in 1669, Sir Issac Newton discovered that light is comprised of different colors as a crude glass prism can fractionate the white light into various colors. He was able to separate all the colors and created the visible spectrum of sunlight through prism, which are the same as found in the rainbow. However, since UV rays are undetected by naked eye, it was not until another 107 years that Johann Wilhelm Ritter from Jena in Germany discovered the UV component of solar spectrum (Barth et al. 1987). In 1801, Ritter was able to demonstrate that the invisible component of sunlight lower than the visible violet end of solar spectrum causes the strongest chemical reaction on silver chloride and called it ultraviolet rays. He also discovered infrared rays on the other side of visible spectrum by showing that blackening effect did not occur by blocking just visible spectrum (Herschel 1800). However, nobody understood the importance of his discovery and it was passed nearly unnoticed. It was only after a gap of about 140 years, during World War II, that the sun was recognized as a source of radio waves and ionizing radiation on the earth (Menzel 1959).

Ultraviolet light is a type of electromagnetic radiation. The primary source of electromagnetic radiation is the sun. They are transmitted in the form of waves or particles at varying wavelengths and frequencies, creating the broad range of wavelengths collectively known as the electromagnetic (EM) spectrum. The EM spectrum is arranged into seven regions with decreasing wavelength and increasing energy and frequency. These seven regions of EM spectrum are popularly known as radio waves, microwaves, infrared, visible light, ultraviolet (UV), X-rays, and gamma rays. UV wavelength range from 200 to 400 nm falls between visible light and X-rays under EM spectrum. The range from 200 to 400 nm is further broken down arbitrarily into UV-A, UV-B, and UV-C radiations, based on skin reactions in human beings. The UV-A portion has the longer wavelength range from 320 to 400 nm and does not fall in the absorption range of proteins and nucleic acids. It is harmless to skin at moderate exposure or in the absence of photosensitizing chemicals. UV-A wavelength range is also referred to as black light and near-UVR. UV-B radiation wavelength range is from 290 to 320 nm and falls under absorbance range of proteins and nucleic acids. It is erythema on exposed skin and is present in the terrestrial solar spectrum. It is also called as sunburn radiation and midrange UVR. UV-C radiation wavelength range is from 200 to 290 nm and is biologically active. It is completely absorbed by ozone layer and does not reach the earth's surface. However, because of its germicidal properties, it is frequently generated artificially at the 254 nm wavelength in low-pressure mercury lamps (germicidal lamps) for use in experiments.

Biologically important effects of solar radiation are due to wavelength in the 290–106 nm range, except in special circumstances such as photo-drug reactions and certain disease states. Visible light of the EM spectrum is considered harmless and rather essential for normal individuals. Infrared is essentially heat and provides warmth. It is still unknown at present if infrared radiation has a role in skin cancer

(Lydahl 1984; Jones et al. 1988; Akasaka and Kon 1989). Thus, so far the major source of the damaging effects of sunlight is UV portion of the spectrum between 290 and 400 nm (Kligman 1982, 1986).

1.2 Skin and UVR

Despite the unquestioned importance of solar radiation for human survival, most evidence to date indicates that sunlight is predominantly toxic to human skin. One exception is the important role played by sunlight in vitamin D metabolism in the skin. Rickets was observed in population living in areas of less sun exposure. Some investigators have suggested that racial patterns of skin pigmentation (melanogenesis) vary inversely with need for UV light exposure for vitamin D production (Loomis 1967).

UV-B radiation helps making vitamin D and can also inhibit cell mitosis as well as induces sunburn. The UV-A radiation is responsible for majority of known phototoxic and photoallergic reactions. Longer wavelength of UV-A (UV-A-I, 340–400 nm) is less detrimental than the shorter UV-A wavelength (UV-A-II, 320–340 nm) (NIHCDCS 1989). UV-B exposure was considered as the main cause of photo-induced skin cancer but prolonged UV-A exposure can also cause skin cancer (Sterenborg and Van der Leun 1990). UVR and other ionizing radiations induce double-strand DNA breaks in human epithelioid cells (Peak and Peak 1990). UV-A evokes a biphasic erythema response as well as DNA and cell membrane damage (Chew et al. 1987, 1988). Light of wavelengths between 250 and 3000 nm enters the outermost layer of the skin. The stratum corneum layer of skin itself reflects approximately 4–7% of UV radiation because of differences in refractive index between air and stratum corneum (Anderson and Parrish 1981). Further attenuation of light, specifically shorter UV wavelengths, is carried out in the stratum corneum due to absorption by chromatophores such as urocanic acid, melanin, and proteins containing aromatic amino acids (tryptophan and tyrosine). About 40% of UV-B pass through and get transmitted to the viable epidermis (Everett et al. 1966).

Light gets transmitted to the dermis mainly due to scattering. Visible light get significantly absorbed by melanin, carotenoids, bilirubin, hemoglobin, and oxyhemoglobin. Longer wavelengths are more penetrative while shorter wavelengths lower than 400 nm get extensively absorbed while passing through different layers of the skin. The amount of melanin present in skin affects the amount of light that gets attenuated, particularly in shorter wavelength range between 300 and 400 nm. Dark-skinned individuals have 1.5 times more absorption than Caucasians (Pathak 1967). Capillary vessels get access close to skin's surface because of papillary boundaries in between the epidermis and dermis. This allows blood and other components present in blood get exposed to the light. In an adult human being, within 20 min an equivalent of whole blood volume passing through skin can potentially get irradiated. Ocular injury and aging of the eye can also result from exposure to radiant energy

due to oxidative stress. Interestingly, because of the presence of various molecular species in the eye, different parts of the eye are selective for absorption of wavelengths. The cornea absorbs UV-B, lens absorbs UV-A with some UV-B, and retina and pigment epithelium absorbs the blue light (Zigman 1993).

1.3 UVR and Aging

UV exposure drastically affects skin with almost 80% of visible signs of aging in the skin including dry appearance, scalping, wrinkling (Grant 2008) and impaired pigmentation, and photoaging. Skin aging also correlates with cancer risk. As per a 2012 study of Central Europeans, early wrinkling on the neck skin in people is linked with four times more susceptibility to melanoma compared to general population. Similarly, people with freckled skin on the back showed more than three times the risk (Wendt et al. 2012). Skin aging and risk of melanoma are both correlated with age and amount of UV exposure. Melanoma usually occurs later in the life with average age at the time of diagnosis about 55 with five to over 60 cases per 100,000 people per year worldwide (Garbe and Leiter 2009). Although melanoma mostly occurs at the age of 50–60 years, 20% of the cases occur in young adults (Garbe and Orfanos 1990; Bishop et al. 2007). Noticeably, accumulation of mutations caused by UV-induced DNA damage which is the major factor for melanoma formation begins with sun exposure early in life; therefore, sun protection in the childhood is very important. Melanoma risk is significantly correlated with excessive sun exposure in the early years of life. Structural anatomical differences in children skin compared to adults make it easier for UV penetration (Volkmer and Greinert 2011). Excessive exposure to UV radiation in childhood increases the risk of melanoma in young adults under the age of 30 by over three times. This informs that excessive UV exposure can accelerate the initiation of carcinogenesis (Cust et al. 2011). Furthermore, according to a detailed study conducted in Sweden on over three million people, UV damage starts accumulating as early as in the neonatal age. The study also showed that melanoma incidence is more in those born in spring and summer *compared to* those born in fall or winter (Crump et al. 2014). Indeed, it is estimated that 80% of lifetime UV exposure occurs before the age of 20 because of more outdoor recreational activities.

In past few decades, melanoma risk has increased among middle-aged population. According to an epidemiologic study in Minnesota, there is 24-fold increased risk among population with 60 cases per 100,000 in 2009 compared to just eight per 100,000 in 1970. Another finding suggests the unfortunate steady increase in incidence among young adults especially young women in the United States (US). For young American women aged 15–39, incidences of melanoma have increased twofold with 14 out of 100,000 cases per year in 2006 compared to 6 out of 100,000 cases in 1973 (Purdue et al. 2008). The main reason for increased incidences of melanoma among younger population is the increased use of artificial tanning UV sources (Bishop et al. 2007).

2 UVR and Cancer

2.1 Sunburns and Melanoma

Overexposure to UV radiation is the main causing factor for skin cancers, and melanoma incidences correlate with repetitive chronic UV exposures that cause sunburn. Five or more sunburns in a lifetime double the risk for melanoma and sunburns in childhood can increase the risk of melanoma as a young adult (Chang et al. 2009; Cust et al. 2011; Pfahlberg et al. 2001). The link of sunburn with melanoma either suggests the involvement of inflammatory mediators in initiating carcinogenesis or that the melanocytes get transformed with intense UV exposure. Unfortunately, sunburn is common in adults in the US with over half of all adults suffered from it in 2013. Sunburn has widespread presence in over 50% of all adults and over 65% of fair-skinned young adults under the age of 30 in the US population. Furthermore, use of lotions, sprays, and clothing available for sun protection does not help with reducing the prevalence of sunburn (CDC 2012). Various factors contribute to the risk of sunburn including geography, cloud cover, climate, societal norms relating to amount of clothing worn, etc. Negligence from parents regarding sun protection correlates with incidence of sunburns in children (and indoor tanning use among adolescents). Setting up campaigns to educate parents about UV safety might help with prevention and protection against UV-induced skin pathologies (Behrens et al. 2013).

2.2 Skin Pigmentation

Pigmentation of skin is one of the major visible phenotypic traits in humans. Dark pigmentation plays a crucial role in the protection of exposed skin against UV radiation, while in turn UV radiation is responsible for dark skin coloration (Brenner and Hearing 2008). Renato Biasutti, an Italian geographer, was the first one to investigate the association between geographical location and skin pigmentation and created a world map according to phenotypic trait (Barsh 2003; Jablonski and Chaplin 2000). Ethnic populations living near to the equator have the darkest pigmentation with gradual lightening of skin color with increase in latitude and moving away from equator. However, human pigmentation pattern does vary among northern and southern hemispheres because of differences in UV radiation. Other hypotheses are that the fair skin complexion is a positive adaptation to maximize UV-induced vitamin D synthesis in climates with limited sun exposure while on the other hand dark skin color near equator is selected to protect against strong UV radiation. Sometimes, light-colored skin arises due to either sexual selection or relaxation of selection.

Skin pigmentation comes from melanin which is synthesized in an enzymatically controlled manner (Simon et al. 2009). Melanin exists in two different forms, eumelanin and pheomelanin. Eumelanin is a dark black insoluble polymer while pheomelanin is a light red-yellow sulfur-containing soluble polymer. Both eumelanin and pheomelanin are indole derivatives of 3,4 dihydroxyphenylalanine (DOPA). The

enzyme tyrosinase catalyzes a critical rate-limiting step of tyrosinase oxidation to dopaquinone, a precursor of L-DOPA. Various melanocyte-specific enzymes, including tyrosine-related proteins TYRP1 and TYRP2 (also known as dopachrome tautomerase, DCT), cause subsequent metabolism of DOPA and its derivatives resulting in synthesis of eumelanin. The synthesis of pheomelanin involves production of cysteinyldopa through conjugation of dopaquinine by thiol-containing cysteine or glutathione (Yamaguchi et al. 2007). In vitro studies suggest that melanogenesis occurs in three distinct steps. First step is the production of cysteinyldopa which is then oxidized to form pheomelanin. Final step is the production of eumelanin which is deposited on the preformed pheomelanin (Ito 2003). The ratio of the two forms of melanin is maintained through tyrosinase activity and cysteine concentration. The level of tyrosinase activity is dependent on melanosomal pH, which is regulated by cAMP and α-MSH (Cheli et al. 2009). The type and levels of melanin determine the skin complexion and the observed differences in skin color are ultimately caused by variations in the genes involved in pigmentation process.

2.3 Skin Pigmentation and Aging

As people continue to live longer, the incidence of pigmentary changes due to aging will continue to grow. There are many changes that occur in skin pigmentary system associated with age. Although chronological aging-related changes are inevitable, some conditions like photoaging are preventable. Pigmentary changes are generally considered as harmless cosmetic problem. However, some of those conditions are cosmetic but can also be premalignant and malignant.

Skin aging is a basic biologic process resulting from genetic programming or intrinsic aging as well as cumulative environmental damage. Intrinsic aging is universal and inevitable as it is attributed to the passage of time. Photoaging is caused by cumulative effects of prolonged sun exposure coupled with intrinsic aging. It results in skin damage and induction of inflammatory response.

Several pigmentary disorders are associated with the aging process. The amount of melanin may increase or decrease with advancing age. Approximately 1–2% of all epidermal cells are melanocytes, cells that synthesize melanin. Melanocyte density is about two times higher in sun-exposed skin compared to unexposed skin. The number of melanocytes decreases by 8–20% per decade after age 30 in both sun-exposed and sun-unexposed skin. Repeated exposure of UV radiation stimulates melanocyte growth through upregulation of interleukin 1, interleukin 6, interleukin 8, tumor necrosis factor α, transforming growth factor β, basic fibroblastic growth factor, endothelin derivatives, and nerve growth factor, the last of which is secreted by keratinocytes. Keratinocyte gives rise to molecules that have the direct effect on melanocyte proliferation and survival. These molecules may also play a role in the pathogenesis of dyspigmentation of photoaged skin. Inflammatory mediators like leukotriene C1 stimulate melanocyte growth and amend their phenotype, which may help explain melanocyte oncogenesis.

Melanocytes of photoaged skin have many structural changes like nuclear heterogeneity, abundant cytoplasmic organelles, and elongation of dendrites. Signs of increased cell activity such as increased melanogenesis and large melanosomes are also seen in photoaged skin. Sun-exposed melanocytes also go through significant cytomorphometric changes such as decreased cell and nuclear size, increased cell and nuclear perimeters, and higher degrees of nuclear ellipticity.

Commonly seen pigmentary changes because of age are pigmented growths on the face, neck, and body. Seborrheic keratoses (SKs) and dermatosis papulosa are few of the most common problems. SK is a benign neoplasm that starts appearing between the age of 30s and 50s and increases in size and number with age. SKs are considered the best biomarker for intrinsic aging of skin. Dermatosis papulosa nigra is a condition of multiple, 1–5 mm dark brown flat-topped papules on the face and neck. It is a common condition in African American populations, with approximately 77% of adults affected by the age of 10–42 years. It is most likely hereditary since 54% of patients have a family history of similar lesions. Both the number and size of individual lesions increase with advancing age.

Melanocytic nevi lesions occur in childhood and adolescence, reaching peak in the second and third decade. The number of lesions decreases with increasing age and rarely found in individuals older than 80, replaced by connective tissue elements.

Gray hairs are also a common phenomenon with nearly half of the population by the age of 50 having at least 50% gray scalp hair. Graying of hairs is due to progressive loss of melanocytes present in hair bulb. Follicular melanocytes in hair get depleted more rapidly compared to skin melanocytes probably due to exhaustion because of excessive proliferation and melanin synthesis during anagen phase of the hair cycle.

All races are more or less susceptible to photoaging. However, Fitzpatrick skin phototypes IV to VI in people are less susceptible, which may be due to the presence of more photo-protective melanin. In African Americans, lighter-skinned individuals are more prone to photoaging with apparent signals that start in the fifth or sixth decade of life. Mottled, irregular dyspigmentation is a common symptom of photoaged skin of all races; however, mechanisms driving mottled pigmentation are unknown. Microscopically, there is uneven distribution of melanocytes with heterogenous distribution of melanosomes within keratinocytes. Photoaging of skin also causes both benign hyper- and hypopigmented lesions and malignant lesions.

Another very common phenomenon is idiopathic guttate hypomelanosis, which is an acquired hypopigmentary disorder that usually develops after the age of 40 or 50 and increases with age. Small white patch or plaque commonly found in elderly individuals with fair complexion is called as spontaneous stellate pseudoscars. Solar lentigo is one of the most common benign sun-induced lesions which increases with age. Sun-exposed skin of elderly patients has lentigo maligna which is distinguishable as an asymmetrical area of homogenous pigmentation ranging from dark brown to black with irregular border. The incidence of developing lentigo maligna increases progressively with age, with an average age of onset of 65 years.

2.4 Role of Pigmentation in Cancer

Skin pigment melanin absorbs sunlight and protects against sunlight-induced burns, DNA damage, and skin cancer. Melanin has an unusually broad absorption spectrum and perhaps also has radical scavenging activity (Kollias et al. 1991). Yet there are discrepancies in literature about the protective role of melanin. Blondes and redheads with higher ratio of yellow pheomelanin compared to brown eumelanin in skin and hairs have twofold to fourfold greater risk of melanoma than dark-haired individuals (Williams et al. 2011). Mice lacking melanin do not form melanoma after UV-A irradiation (Noonan et al. 2012), and Braf mutant mice with pheomelanin-associated $Mc1r^{e/e}$ allele develop ten times more spontaneous melanomas (Mitra et al. 2012). UV-sensitized melanin, especially pheomelanin, triggers apoptosis and production of reactive oxygen species (ROS) and DNA strand breaks (Chedekel et al. 1978; Takeuchi et al. 2004). Melanin synthesis itself generates ROS, especially pheomelanin synthesis (Munoz-Munoz et al. 2009). Melanin-induced ROS generation is not solely responsible for melanoma development. Majority of mutations in human melanomas have the UV signature of C>T substitutions at sites of adjacent pyrimidines (Brash 2015; Krauthammer et al. 2012). Such mutations occur from the formation of cyclobutane pyrimidine dimers (CPDs) by joining of adjacent pyrimidines to distort the DNA helix (Brash et al. 1987; Schreier et al. 2007). Genetic disorders of CPD repair, such as in xeroderma pigmentosum, increase the risk for childhood melanoma by four orders of magnitude.

Premi et al. (2015) have experimentally shown that melanin can be carcinogenic to cells. UV radiation exposure of melanin-containing cells causes induction of superoxide and nitric oxide, causing a factor of ~400 peroxynitrite spike that degrades melanin. Even hours after initial UV exposure, excited melanin derivatives exist in a triplet state with high energy of UV photon. These excited derivatives go to the nucleus and transfer their energy to DNA causing mutagenic cyclobutene pyrimidine dimes in the dark ultimately leading to cancer-causing mutations.

3 Conclusion

Overall, solar radiation reaching the earth's surface is an inherent part of our life and is necessary for our day-to-day activities as well as survival. Exposure to sun is almost unavoidable. Although our body has natural protection because of skin and its pigmentation, repeated exposure can overcome that with time and will lead to more detrimental effects. Aging of skin is an autonomous process and will happen with time but excessive exposure to sun can anticipate the process leading to premature skin aging and cancer. Cancer is one of the most common and lethal effects of sun exposure and can be avoidable by minimum exposure, proper clothing, and sunscreens.

References

Akasaka, T., & Kon, S. (1989). Two cases of squamous cell carcinoma arising from erythema abigne. *Nippon Hifuka Gakkai Zasshi. Japanese Journal of Dermatology, 99*, 735–742.

Anderson, R. R., & Parrish, J. A. (1981). The optics of human skin. *Journal of Investigative Dermatology, 77*, 13–19. https://doi.org/10.1111/1523-1747.ep12479191.

Barsh, G. S. (2003). What controls variation in human skin color? *PLoS Biology., 1*, E27. https://doi.org/10.1371/journal.pbio.0000027.

Barth, J., Johnson, W., & Entdeekung, D. (1987). No Title. *Jahren. Hautarzt, 38*, 301–303.

Behrens, C. L., Thorgaard, C., Philip, A., & Bentzen, J. (2013). Sunburn in children and adolescents: Associations with parents' behaviour and attitudes. *Scandinavian Journal of Public Health, 41*, 302–310. https://doi.org/10.1177/1403494813476158.

Bishop, J., Bataille, V., Gavin, A., Lens, M., Marsden, J., & Mathews, T. W. C. (2007). The prevention, diagnosis, referral and management of melanoma of the skin: Concise guidelines. *Clinical Medicine (London), 7*(3), 283–290.

Brash, D. E. (2015). UV signature mutations. *Photochemistry and Photobiology., 91*, 15–26. https://doi.org/10.1111/php.12377.

Brash, D. E., Seetharam, S., Kraemer, K. H., Seidman, M. M., & Bredberg, A. (1987). Photoproduct frequency is not the major determinant of UV Base substitution hot-spots or cold spots in human-cells. *Proceedings of the National Academy of Sciences of the United States of America, 84*, 3782–3786. https://doi.org/10.1073/pnas.84.11.3782.

Brenner, M., & Hearing, V. J. (2008). The protective role of melanin against UV damage in human skin. *Photochemistry and Photobiology., 84*, 539–549. https://doi.org/10.1111/j.1751-1097.2007.00226.x.

CDC. (2012). Sunburn and sun protective behaviors among adults aged 18–29 years--United States, 2000–2010. *MMWR Morbidity and Mortality Weekly Report, 61*(18), 317–322. https://doi.org/10.9789/2175-5361.2014v6n2p651.

Chang, Y. M., Barrett, J. H., Bishop, T. D., Armstrong, B. K., Bataille, V., Bergman, W., et al. (2009). Sun exposure and melanoma risk at different latitudes: A pooled analysis of 5700 cases and 7216 controls. *International Journal of Epidemiology, 38*, 814–830. https://doi.org/10.1093/ije/dyp166.

Chedekel, M. R., Smith, S. K., Post, P. W., Pokora, A., & Vessell, D. L. (1978). Photodestruction of pheomelanin: Role of oxygen. *Proceedings of the National Academy of Sciences, 75*, 5395–5399. https://doi.org/10.1073/pnas.75.11.5395.

Cheli, Y., Luciani, F., Khaled, M., Beuret, L., Bille, K., Gounon, P., et al. (2009). αMSH and cyclic AMP elevating agents control melanosome pH through a protein kinase A-independent mechanism. *Journal of Biological Chemistry, 284*, 18699–18706. https://doi.org/10.1074/jbc.M109.005819.

Chew, S., Deleo, V. A., & Harber, L. C. (1987). An animal model for evaluation of topical photoprotection against ultraviolet a (320–380 nm) radiation. *Journal of Investigative Dermatology, 89*, 410–414. https://doi.org/10.1111/1523-1747.ep12471776.

Chew, S., Deleo, V. A., & Harber, L. C. (1988). Longwave ultraviolet radiation (UVA)-induced alteration of epidermal DNA synthesis. *Photochemistry and Photobiology, 47*, 383–389. https://doi.org/10.1111/j.1751-1097.1988.tb02741.x.

Crump, C., Sundquist, K., Sieh, W., Winkleby, M. A., & Sundquist, J. (2014). Season of birth and other perinatal risk factors for melanoma. *International Journal of Epidemiology, 43*, 793–801. https://doi.org/10.1093/ije/dyt277.

Cust, A. E., Jenkins, M. A., Goumas, C., Armstrong, B. K., Schmid, H., Aitken, J. F., et al. (2011). Early-life sun exposure and risk of melanoma before age 40 years. *Cancer Causes and Control, 22*, 885–897. https://doi.org/10.1007/s10552-011-9762-3.

Everett, M. A., Yeargers, E., Sayre, R. M., & Olson, R. L. (1966). Penetration of epidermis by ultraviolet rays. *Photochemistry and Photobiology, 5*, 533–542. https://doi.org/10.1111/j.1751-1097.1966.tb09843.x.

Garbe, C., & Leiter, U. (2009). Melanoma epidemiology and trends. *Clinics in Dermatology, 27*, 3–9. https://doi.org/10.1016/j.clindermatol.2008.09.001.

Garbe, C., & Orfanos, C. E. (1990). Epidemiology of malignant melanoma in Central Europe: Risk factors and prognostic predictors. Results of the central malignant melanoma registry of the German dermatological society. *Pigment Cell Research, 3*, 285–294. https://doi.org/10.1111/j.1600-0749.1990.tb00387.x.

Grant, W. B. (2008). The effect of solar UVB doses and vitamin D production, skin cancer action spectra, and smoking in explaining links between skin cancers and solid tumours. *European Journal of Cancer, 44*, 12–15. https://doi.org/10.1016/j.ejca.2007.09.009.

Herschel, W. (1800). Investigation e powers of the prismatic colours etc. *Philosophical Transactions*, 255–292.

Ito, S. (2003). IFPCS presidential lecture: A chemist's view of melanogenesis. *Pigment Cell Research, 16*, 230–236. https://doi.org/10.1034/j.1600-0749.2003.00037.x.

Jablonski, N. G., & Chaplin, G. (2000). The evolution of human skin coloration. *Journal of Human Evolution, 39*, 57–106. https://doi.org/10.1006/jhev.2000.0403.

Jones, C., Tyring, S., Lee, P., & Fine, J. (1988). Development of neuroendocrine (Merkel cell) carcinoma mixed with squamous cell carcinoma in erythema abigne. *Archives of Dermatology, 124*, 110–113.

Kligman, L. (1982). Intensification of ultraviolet-induced dermal damage by infrared radiation. *Archives of Dermatological Research, 272*, 229–238.

Kligman, L. (1986). Photoaging. Manifestations, prevention, and treatment. *Dermatologic Clinics, 4*, 517–528.

Kollias, N., Sayre, R. M., Zeise, L., & Chedekel, M. R. (1991). Photoprotection by melanin. *Journal of Photochemistry and Photobiology B, 9*, 135–160. https://doi.org/10.1016/1011-1344(91)80147-A.

Krauthammer, M., Kong, Y., Ha, B. H., Evans, P., Bacchiocchi, A., McCusker, J. P., et al. (2012). Exome sequencing identifies recurrent somatic RAC1 mutations in melanoma. *Nature Genetics, 44*, 1006–1014. https://doi.org/10.1038/ng.2359.

Loomis, W. F. (1967). Skin-pigment regulation of vitamin-D biosynthesis in man. *Science, 157*, 501–506. https://doi.org/10.1126/science.157.3788.501.

Lydahl, E. (1984). Infrared radiation and cataract. *Acta Ophthalmologica – Supplementum, 166*, 1–63.

Menzel, D. (1959). *Our Sun, rev*. Cambridge, MA: Harvard University Press.

Mitra, D., Luo, X., Morgan, A., Wang, J., Hoang, M. P., Lo, J., et al. (2012). An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. *Nature, 491*, 449–453. https://doi.org/10.1038/nature11624.

Munoz-Munoz, J. L., García-Molina, F., Varón, R., Tudela, J., García-Cánovas, F., & Rodríguez-López, J. N. (2009). Generation of hydrogen peroxide in the melanin biosynthesis pathway. *Biochimica et Biophysica Acta – Proteins and Proteomics, 1794*, 1017–1029. https://doi.org/10.1016/j.bbapap.2009.04.002.

NIHCDCS. (1989). Sunlight, ultraviolet radiation, and the skin. 7(8).

Noonan, F. P., Zaidi, M. R., Wolnicka-Glubisz, A., Anver, M. R., Bahn, J., Wielgus, A., et al. (2012). Melanoma induction by ultraviolet A but not ultraviolet B radiation requires melanin pigment. *Nature Communications, 3*, 884. https://doi.org/10.1038/ncomms1893.

Pathak, M. (1967). Photobiology of melanogenesis: Biophysical aspects. In W. Montagna & F. Hu (Eds.), *Advances of biology of skin*. New York: Pergamon Press.

Peak, J. G., & Peak, M. J. (1990). Ultraviolet light induces double-strand breaks in DNA of cultured human P3 cells as measured by neutral filter elution. *Photochemistry and Photobiology, 52*, 387–393. https://doi.org/10.1111/j.1751-1097.1990.tb04194.x.

Pfahlberg, A., Kölmel, K. F., & Gefeller, O. (2001). Timing of excessive ultraviolet radiation and melanoma: Epidemiology does not support the existence of a critical period of high susceptibility to solar ultraviolet radiation-induced melanoma. *British Journal of Dermatology, 144*, 471–475. https://doi.org/10.1046/j.1365-2133.2001.04070.x.

Premi, S., Wallisch, S., Mano, C. M., Weiner, A. B., Bacchiocchi, A., Wakamatsu, K., Bechara, E. J. H., Halaban, R, Douki, T., & Brash, D. E. (2015). Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure. Science, 347(6224), 842–847. https://doi.org/10.1126/science.1256022.

Purdue, M. P., Freeman, L. E. B., Anderson, W. F., & Tucker, M. A. (2008). Recent trends in incidence of cutaneous melanoma among US caucasian young adults. *Journal of Investigative Dermatology*. https://doi.org/10.1007/s10562-017-1994-8.

Schreier, W. J., Schrader, T. E., Koller, F. O., Gilch, P., Crespo-Hernández, C. E., Swaminathan, V. N., et al. (2007). Thymine dimerization in DNA is an ultrafast photoreaction. *Science, 315*, 625–629. https://doi.org/10.1126/science.1135428.

Simon, J. D., Peles, D., Wakamatsu, K., & Ito, S. (2009). Current challenges in understanding melanogenesis: Bridging chemistry, biological control, morphology, and function. *Pigment Cell and Melanoma Research, 22*, 563–579. https://doi.org/10.1111/j.1755-148X.2009.00610.x.

Sterenborg, H. J. C. M., & van der Leun, J. C. (1990). Tumorigenesis by a long wavelength UV-A source. *Photochemistry and Photobiology, 51*, 325–330. https://doi.org/10.1111/j.1751-1097.1990.tb01718.x.

Takeuchi, S., Zhang, W., Wakamatsu, K., Ito, S., Hearing, V. J., Kraemer, K. H., & Brash, D. E. (2004). Melanin acts as a potent UVB photosensitizer to cause an atypical mode of cell death in murine skin. *Proceedings of the National Academy of Sciences, 101*, 15076–15081. https://doi.org/10.1073/pnas.0403994101.

Volkmer, B., & Greinert, R. (2011). UV and children's skin. *Progress in Biophysics and Molecular Biology, 107*, 386–388. https://doi.org/10.1016/j.pbiomolbio.2011.08.011.

Wendt, J., Schanab, O., Binder, M., Pehamberger, H., & Okamoto, I. (2012). Site-dependent actinic skin damage as risk factor for melanoma in a central European population. *Pigment Cell and Melanoma Research, 25*, 234–242. https://doi.org/10.1111/j.1755-148X.2011.00946.x.

Williams, P. F., Olsen, C. M., Hayward, N. K., & Whiteman, D. C. (2011). Melanocortin 1 receptor and risk of cutaneous melanoma: A meta-analysis and estimates of population burden. *International Journal of Cancer, 129*, 1730–1740. https://doi.org/10.1002/ijc.25804.

Yamaguchi, Y., Brenner, M., & Hearing, V. J. (2007). The regulation of skin pigmentation. *Journal of Biological Chemistry, 282*, 27557–27561. https://doi.org/10.1074/jbc.R700026200.

Zigman, S. (1993). Ocular light damage. *Photochemistry and Photobiology, 57*, 1060–1068. https://doi.org/10.1111/j.1751-1097.1993.tb02972.x.

UVR and Vitamin D Synthesis

Deepti Chopra, Dhanananajay Kumar, Divya Dubey, Jyoti Singh, Ajeet Kumar Srivastav, and Kailash Chand Gupta

Abstract

Vitamin D is also known as the sunshine vitamin for all the good reasons. During the skin exposure to sunlight, UVB photolyses 7-dehydrocholesterol of skin into pre-vitamin D3 which further gets isomerized into vitamin D3. Human beings typically count on sunlight exposure for their total vitamin D need. Skin pigmentation/colouration, use of proper sunscreen, photoaging of one's skin, particular time of the day, seasonal changes/fluctuations and, lastly, latitude hugely influence synthesis of pre-vitamin D3. Around 50% of total population of the world is currently under deficiency of vitamin D according to reports. Vitamin D deficiency may be partially due to inadequate vitamin D-enriched foods and well-known misapprehension of healthy diet being adequate in vitamin D. Its deficiency is responsible for causing retardation in growth and rickets primarily

D. Chopra · D. Dubey · A. K. Srivastav
Photobiology Laboratory, Systems Toxicology and Health Risk Assessment Group, CSIR-Indian Institute of Toxicology Research, Lucknow, India

Babu Banarasi Das University, Lucknow, India

D. Kumar
Department of Pharmaceutical Engineering & Technology, IIT-BHU, Varanasi, Uttar Pradesh, India

J. Singh
Photobiology laboratory, Systems Toxicology and Health Risk Assessment Group, CSIR-Indian Institute of Toxicology Research, Lucknow, India

Academy of Scientific and Innovative Research, New Delhi, India

K. C. Gupta (✉)
CSIR-Institute of Genomics and Integrative Biology, New Delhi, India

A. Dwivedi et al. (eds.), *Skin Aging & Cancer*,
https://doi.org/10.1007/978-981-13-2541-0_7

in kids. Further, in adults it may cause osteoporosis, osteopenia and increased risk of fractures. The other severe consequences include cardiovascular disease, infectious diseases, autoimmune diseases and common cancers. Thus, for the humans, there is a constant need to emphasize on the intake of crucial amount of sunlight to fulfil their essential threshold vitamin D requirements for a better well-being.

Keywords
Vitamin D · Autoimmune diseases · Osteoporosis

1 Introduction

The best-known foods rich in vitamin D are mushrooms, eggs, fatty fishes (eel, salmon, tuna and sardines) and fish oils (cod liver oil). In the USA, various food items like milk, soy milk and cereal grains come with pre-added vitamin D. There is a close association between exposure of ultraviolet radiation (UVR) and vitamin D production. Plentiful researches have shown a comprehensible link between sunlight and vitamin D synthesis in our bodies. Therefore sun exposure is needed to synthesize an adequate amount of vitamin D for paramount health of people. Similarly, its deficiency may occur due to too little vitamin D in food and drinks, lesser time spent outdoors in sunlight and diverse health anomalies that involve assimilation/alteration of this vitamin inside the body.

A few people only take adequate vitamin D according to one's requirements, and so most of the population needs some minimal sunlight to enhance their vitamin D levels, necessary for a better well-being. Vitamin D is synthesized in the skin when UVB radiations react with 7-dehydrocholesterol (Olds et al. 2008). The most appropriate wavelength for UV-mediated synthesis ranges between 270 and 300 nm. These wavelengths correspond to UV index of more than three values.

The time duration an individual requires to make an adequate amount of vitamin D for a good health relies on the placement from the equator, season and melanin content in the skin; i.e. fair skin requires lesser time than darker skin.

Most individuals will have sufficient levels of vitamin D just by minor exposure, yet out of the peak UVR times between 11 am and 3 pm. It is thoughtful that only 5 min of minor sun exposure daily is adequate enough for an individual who easily burns, while up to 20 min is sufficient for a one with darker skin. Globally, sun is the prime source of vitamin D and also depends on vitamin D levels present initially. At higher altitudes, cold temperature and reduced solar intensity diminish skin's sunlight exposure in the populations; therefore, the type of food intake becomes a crucial source of vitamin D in them (Engelsen 2010).

2 Photoproduction of Vitamin D3

During sunlight exposure, UVB gets absorbed by 7-dehydrocholesterol of the epidermis and dermis of the skin. This absorption then causes double bonds excitation to open B-ring to form a flexible molecule, pre-vitamin D3. Pre-vitamin D3 generally has two conformations. Among the two, cis-form being thermodynamically less favourable gets converted into vitamin D3. The integration of 7-dehydrocholesterol in fatty acid hydrocarbon side chain and polar head group of triglycerides in the plasma membrane augments thermal-induced isomerization of pre-vitamin D3 to vitamin D3. On sunlight exposure, 7-dehydrocholesterol gets converted to cis-form which then isomerizes into vitamin D3. Vitamin D3 gets ejected out of plasma membrane into extracellular space, where it enters dermal capillary bed bound to vitamin D binding protein.

It has been quite debatable as to whether dietary vitamin D3 is comparable to skin-generated vitamin D3. Though both dietary and the skin-generated one have the similar biological actions, but as they get metabolized, the half-life of skin-generated vitamin D3 gets prolonged in the circulation as all of it is bound to vitamin D binding protein. In the case of dietary vitamin D3, 60% of total is bound to vitamin D binding protein; furthermore 40% gets quickly cleared in the lipoprotein-bound fraction.

3 Factors Responsible for the Synthesis of Cutaneous Vitamin D

Melanin is a known sunscreen that absorbs UVA (320–400 nm) and UVB (280–320 nm) radiations, thereby protecting the cellular UV-absorbing biomolecules, viz. DNA, RNA and proteins from the phototoxic effects of UVR. Nevertheless, as the population drifted towards north and south regions of the equator, a prompt mutation in their skin pigment gene is needed to sustain their ability to make adequate vitamin D3 for normal calcium as well as bone metabolism. Melanin is consequently competent in quenching UVB radiation that it noticeably diminishes the photoproduction of cutaneous vitamin D3. The melanin (dark) pigment (Africans and African-Americans) having skin types 5 and 6 (only tans) very well absorbs UVB radiation and lessens the ability of skin for pre-vitamin D3 (95–99%) production, in comparison to Caucasians with skin type 2 (sometimes tans, burns mostly) (Shaw and Pal 2002). Similarly a sunscreen application with a sun's protection factor 15 absorbs UVB radiation (approximately 99%) and, accordingly, reduces cutaneous pre-vitamin D3 production by 99%. The angle at which the sunlight hit the earth's surface also has a remarkable effect on the cutaneous photoproduction of pre-vitamin D3. Thus, seasonal variations, latitudes, time of the day and the weather

conditions dramatically affect the cutaneous photoproduction of pre-vitamin D3. Existing above and below in the region of 35° latitude, children and adults can generate an ample amount of vitamin D3 in their skin during the spring, summer and fall. However, fundamentally all of the UVB photons are absorbed during the winter months, thus either eliminating or markedly dipping the capacity of the skin to produce vitamin D3. This is why there is a seasonal variation in circulating levels of 25-hydroxyvitamin D3 [25(OH)D] which is measured to be the chief circulating form of vitamin D. Similarly, in the early morning and late afternoon, as the sun's rays are more oblique, most of the UVB photons get absorbed by the ozone layer.

4 Differences Between Vitamin D Synthesis and Erythema

Vitamin D synthesis and erythema both need unprotected skin exposure to UVR, but have key differences between the two responses. A primary difference is that of the two action spectra. Vitamin D synthesis is a response to UVB radiation (280–315 nm), while erythema is elicited by both UVB and UVA radiations. Erythema is characterized as damage to skin and endpoint of this damage is evident by reddening and blistering of the exposed skin. Vitamin D, on the other hand, is synthesized in the skin, enters the circulation and is hydroxylated in the liver to 25-hydroxyvitamin D (25(OH)D) (Webb 2006). The concentration of circulating 25(OH)D is measured as an indicator of a person's vitamin D condition, as hydroxylation to the active form in kidney, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), is tightly controlled by other factors. Vitamin D synthesized in skin contributes to the concentration of 25(OH)D in the blood, so increasing exposed skin area will directly increase vitamin D level. Other organs also possess receptors for 25(OH)D and the cells can make $1,25(OH)_2D$ for other health benefits. To expand these benefits the circulating 25(OH)D must be elevated than the concentrations required to avoid rickets and the associated vitamin D intake/production must increase likewise.

Erythema occurs when UV exposure exceeds a minimal erythema dose (MED) in a single exposure or exposures collectively. Two sub-erythemal doses in a week time are nonadditive for erythema, while the same two exposures on either side of lunch hour on the same day could produce an erythemal response due to inadequate repair time. All and any exposure may make to cumulative lifetime dose, but avoiding erythema is foremost important in skin cancer prevention and risk reduction for malignant melanoma. The absolute UV dose that generates a slight reddening of the skin, i.e. an MED, is mainly individual dependent. It is broadly associated with skin type and skin colour, but neither is a very exact analyst of MED. The melanin pigment absorbs UVR and prevents it from damaging DNA, or converting 7-dehydrocholesterol to pre-vitamin D in the preliminary step of vitamin D synthesis, although melanin is not the only determinant of MED (Dowdy et al. 2010). The

photochemical production of pre-vitamin D requires adequate UVB photon incident on the skin but is then rapid. The pre-vitamin D can be transformed into numerous other biologically inert isomers and in sunlight, the pre-vitamin D in this isomer mixture never surpasses about 12%. Prolonged exposure is of no benefit further, as there is adequate UVB to produce the initial pre-vitamin D and in the next stage thermal isomerization to vitamin D will take several hours and does not, therefore, remove the pre-vitamin D from the mixture on photochemical timescales (Webb and Engelsen 2008).

A broad-spectrum optimal exposure regime for enough vitamin D maintenance is a sub-erythemal dose of UVB-rich radiation every day or two. The same rule is suitable for avoiding erythema. Regular sun exposure has also shown increased survival in cases of malignant melanoma, an effect that may be mediated by vitamin D.

5 UV Exposure and Adequate Diet: Is It Sufficient for Vitamin D Deficiency?

Vitamin D is a crucial nutrient for maintaining a healthy framework of the body as it forms a vital part of bone metabolism, calcium and phosphorous homeostasis (Holick 1994). There are implications that vitamin D may have numerous other health benefits such as avoidance or mitigation of cancer (Grant et al. 2005), autoimmune diseases (Ponsonby et al. 2005), reduction in hypertension (Pilz et al. 2009) and prevention of influenza (Yamshchikov et al. 2009). Research designates that vitamin D stimulates antimicrobial activity (Liu et al. 2006) and thus may alleviate certain types of infections. There are vitamin D receptors in many organs (Holick 2002), and long-term vitamin D deficiency may provoke an extensive range of harmful biological effects. Global studies have shown that vitamin D status is low across a wide array of populations and age groups at very moderate latitudes (Guillemant et al. 2001, Arya et al. 2004). Extremely low vitamin D levels have been seen in darker skin individuals (Shaw and Pal 2002). Sole vitamin D intake from sunlight and diet are insufficient. Enhanced people awareness along with amended policies in the case of vitamin D can improve public health at relatively reasonable costs. Figure 1 outlines vitamin D synthesis and its metabolism in the body (Holick 2002).

Provitamin D (7-dehydrocholesterol, 7-DHC) is converted to pre-vitamin D in the skin by exposure to UVB radiation. The pre-vitamin D is then isomerized by body heat to form vitamin D3. Vitamin D3 transported by the blood to the liver gets converted to 25-hydroxyvitamin D (25(OH)D). In the kidneys, the formation of the active form of vitamin D, 1,25-dihydroxy vitamin D ($1,25(OH)_2D$), is tightly regulated by the parathyroid hormone (PTH). 1,25-Dihydroxy vitamin D is vital for the uptake of calcium and mobilization of calcium stores.

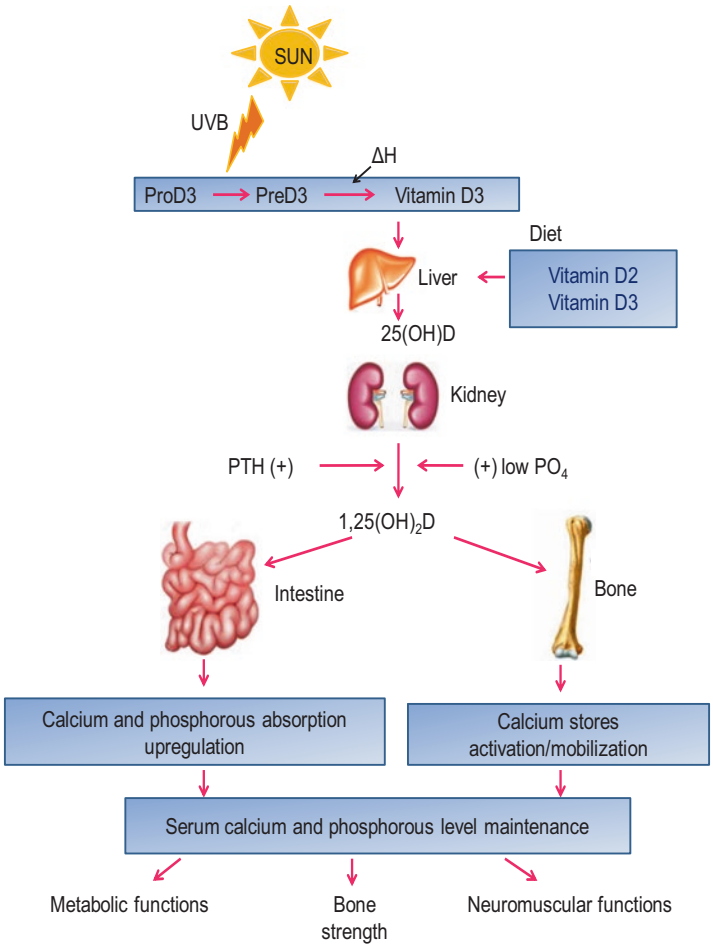

Fig. 1 Outline of vitamin D synthesis and metabolism (Holick 2002)

6 Comparative Effect of Diet and UV Exposure on Serum 25-Hydroxyvitamin D (25(OH)D) Level

Holick and co-workers found that one full-body UV exposure causing a slight pinkness in the skin (one minimum erythemal dose, 1 MED) is equivalent to oral intake in the range 250–625 μg (10,000–25,000 IU) of vitamin D3 (Dowdy et al. 2010). It has been often alleged that sufficient vitamin D is acquired after a few minutes of sunlight exposure. This is usually correct only when the sunlight is high and equivalent of 400 IU (equivalent to one spoonful of cod liver oil) is acquired through exposure. However, many research scientists support higher intake levels of vitamin D (1000–4000 IU). These amounts are typically unavailable from normal daily sun exposure. The gap between beneficial UV exposure to obtain desirable vitamin D

and harmful exposure leading to erythema also narrows i.e. desirable vitamin D effective dose approaches 1 MED (Webb and Engelsen 2014). For example, for normal summer clothing, the exposed skin (25.5%) needs to be sunburnt to produce 4000 IU. The skin would then receive a UV dose associated with elevated skin cancer risk.

7 Conclusion

Human beings have always depended on sunlight for their vitamin D requisite. It is inquisitive that on one hand UVB radiation is so beneficial for vitamin D3 synthesis and on the other hand is the chief cause of non-melanoma skin cancer. The extreme exposure to sunlight and the number of sunburns are mainly accountable for the frightening increase in non-melanoma skin cancer. Vitamin D may pose health implications by location, latitude and time of the year, where quite a few studies have related the development of various diseases, due to vitamin D deficiency, with topography and season. Most experts agree that at least 800–1000 IU of vitamin D/d is needed for all children and adults to help sustain healthy blood levels of 25(OH)D of >30 ng/mL. It is difficult to obtain this amount of vitamin D from dietary sources and also unrealistic to advocate that all human beings on the planet boost their vitamin D intake to 1000 IU of vitamin D/d and also avoid all direct sun exposure. There needs to be moderation in the commendation regarding safe sun exposure related to vitamin D deficiency pandemic. Increased food enrichment with vitamin D and encouraging both children and adults to augment their vitamin D intake from a vitamin D supplement of 1000 IU/d along with sun exposure is needed. The collective effects of UVR and diet on vitamin D status should be investigated more meticulously, both in laboratory environments and at the inhabitant level.

Acknowledgements KC Gupta thanks Indian Council of Medical Research (ICMR), Delhi, India, for awarding Dr. A.S. Paintal Distinguished Scientist Chair at CSIR-IGIB, Delhi. D Chopra thanks Indian Council of Medical Research (ICMR), Delhi, India, for awarding Research Associateship at CSIR-IITR, Lucknow.

References

Arya, V., Bhambri, R., Godbole, M. M., & Mithal, A. (2004). Vitamin D status and its relationship with bone mineral density in healthy Asian Indians. *Osteoporosis International, 15*(1), 56–61.

Dowdy, J. C., Sayre, R. M., & Holick, M. F. (2010). Holick's rule and vitamin D from sunlight. *The Journal of Steroid Biochemistry and Molecular Biology, 121*(1–2), 328–330.

Engelsen, O. (2010). The relationship between ultraviolet radiation exposure and vitamin D status. *Nutrients, 2*(5), 482–495.

Grant, W. B., Garland, C. F., & Holick, M. F. (2005). Comparisons of estimated economic burdens due to insufficient solar ultraviolet irradiance and vitamin D and excess solar UV irradiance for the United States. *Photochemistry and Photobiology, 81*, 1276–1286.

Guillemant, J., Le, H. T., Maria, A., Allemandou, A., Peres, G., & Guillemant, S. (2001). Wintertime vitamin D deficiency in male adolescents: Effect on parathyroid function and response to vitamin D3 supplements. *Osteoporosis International, 12*(10), 875–879.

Holick, M. F. (1994). Vitamin D. In M. Shils, J. A. Olson, & M. Shike (Eds.), *Modern nutrition in health and disease* (pp. 308–325). Malvern: Lea & Febiger.

Holick, M. F. (2002). Vitamin D: The underappreciated D-lightful hormone that is important for skeletal and cellular health. *Current Opinion in Endocrinology & Diabetes, 9*, 87–98.

Liu, P. T., Stenger, S., Li, H., Wenzel, L., Tan, B. H., Krutzik, S. R., Ochoa, M. T., Schauber, J., Wu, K., Meinken, C., Kamen, D. L., Wagner, M., Bals, R., Steinmeyer, A., Zügel, U., Gallo, R. L., Eisenberg, D., Hewison, M., Hollis, B. W., Adams, J. S., Bloom, B. R., & Modlin, R. L. (2006). Toll-Like receptor triggering of a vitamin D-Mediated human antimicrobial response. *Science, 311*, 1770–1773. Nutrients 2010, 2 491.

Olds, W. J., McKinley, A. R., Moore, M. R., & Kimlin, M. G. (2008). In vitro model of vitamin D3 (cholecalciferol) synthesis by UV radiation: Dose–response relationships. *Journal of Photochemistry and Photobiology B: Biology, 93*(2), 88–93.

Pilz, S., Tomaschitz, A., Ritz, E., & Pieber, T. R. M. (2009). Vitamin D status and arterial hypertension: A systematic review. *Nature Reviews. Cardiology, 6*, 621–630.

Ponsonby, A. L., Lucas, R. M., & van der Mei, I. A. (2005). UVR, vitamin D and three autoimmune diseases-multiple sclerosis, type 1 diabetes, rheumatoid arthritis. *Photochemistry and Photobiology, 81*, 1267–1275.

Shaw, N. J., & Pal, B. R. (2002). Vitamin D deficiency in UK Asian families: Activating a new concern. *Archives of Disease in Childhood, 86*(3), 147–149.

Webb, A. R. (2006). Who, what, where and when—Influences on cutaneous vitamin D synthesis. *Progress in Biophysics and Molecular Biology, 92*(1), 17–25.

Webb, A. R., & Engelsen, O. (2008). Ultraviolet exposure scenarios: Risks of erythema from recommendations on cutaneous vitamin D synthesis. In *Sunlight, vitamin D and skin Cancer* (pp. 72–85). New York: Springer.

Webb, A. R., & Engelsen, O. (2014). Ultraviolet exposure scenarios: Risks of erythema from recommendations on cutaneous vitamin D synthesis. *Advances in Experimental Medicine and Biology, 810*, 406–422.

Yamshchikov, A. V., Desai, N. S., Blumberg, H. M., Ziegler, T. R., & Tangpricha, V. (2009). Vitamin D for treatment and prevention of infectious diseases: A systematic review of randomized controlled trials. *Endocrine Practice, 15*, 438–449.

Chemiexcitation of Melanin and Melanoma Pathogenesis

Saroj Kumar Amar and Dhanananajay Kumar

Abstract

Solar radiation is an ultimate source of energy for the existence of life on earth. The UV radiation is a part of solar radiation that acts as a carcinogenic agent by the formation of cyclobutane pyrimidine dimers (CPDs). Earlier studies documented the mechanism of CPD formation in the presence of UV light instantly but recent studies showed the damage of DNA is continuing even after the exposure of UV light till hours. The mechanism of this delayed or dark CPD resembles with chemiluminescence process. The study further detailed the key role of melanocytes in this process of dark CPD formation by chemiexcitation of melanin pigment. Melanin gets excited to higher energy level after UV exposure by the production of free radicals like nitric oxide and superoxide. Thus, this excitation of melanin pigments is responsible factor for damage of DNA after exposure of longer wavelength of UVA which ultimately promotes melanoma pathogenesis.

Keywords

CPD · Chemiexcitation · DNA damage · Melanin · UV radiation

S. K. Amar (✉)
Department of Forensics Science, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

D. Kumar
Department of Pharmaceutical Engineering & Technology, IIT-BHU, Varanasi, Uttar Pradesh, India

A. Dwivedi et al. (eds.), *Skin Aging & Cancer*,
https://doi.org/10.1007/978-981-13-2541-0_8

1 Introduction

Sunlight exposure is widely known environmental nuisance for expansion of cutaneous malignant melanoma (Jhappan et al. 2003). These favor the formation of CPDs (cyclobutane pyrimidine dimers) and pyrimidine (6–4) pyrimidone photoproducts with interactions between UVB and the melanocyte genome. Moreover, UVA composes about 95% of the total UV radiation reaching the earth surface and includes approximately 10–20% of the carcinogenic fraction in sunlight (Valencia and Kochevar 2008). Though longer wavelength of UVA is comparatively lower energetic than UVB but having high penetration power and penetrates till the dermis of the human skin. The popularity of tanning salons has increased the UVA exposure to human beings in recent years and most of the sunscreen lotion contents only UVB filter, thus again exposing with UVA radiation (Haywood et al. 2003).

The role of UVA in cyclobutane pyrimidine dimer formation in mammalian cells has also been documented (Mouret et al. 2006). Prolong exposure of UVA radiation preferentially induced more CPD formation than UVB via a triplet energy transfer mechanism of photosensitization (Mouret et al. 2006). However, it was recently reported in the science that over half of the CPDs in skin arise from a slow process in which UVA or UVB exposure triggers enzymatic events that continue for hours after UV ends, chemically exciting an electron of pigmented melanin to a quantum triplet state ("chemiexcitation") which has the same high energy as a UV photon (Premi et al. 2015). This energy rapidly transfers to DNA, making CPDs without photons.

Chemical excitation of an electron to a high-energy quantum triplet state starkly contrasts with ordinary chemistry, in which molecular collisions excite to rotational or vibrational states to manyfold lower energies. It deals numerous checkpoints for preventing the formation of dark CPDs: by blocking enzyme activation, quenching reactive oxygen and nitrogen species and quenching triplet state energy earlier, it can transfer to DNA. Chemiexcitation also proposes fresh roots for peculiar differences in melanoma risk, afar skin darkness and DNA repair: polymorphisms in photoinduced signaling, enzyme induction, scavenging systems, chemical reactivity of diverse melanin types and inherent triplet state quenchers. An unusual asset of excited state has been reported (Turro et al. 2010). Apart from high energies, the shapes of the molecule's electron orbitals are changing radically, changing the spatial constraints on reactions and accepting reactions that cannot take place at room temperature. In addition, only one of the two oppositely spinning electrons in an electron pair is excited; the now-unpaired electrons resemble two free radicals, which are chemically reactive.

An unexpected finding was that melanocytes were more resistant to direct UVA oxidative stress and the least efficient generator of bystander signaling while they were also paradoxically the most vulnerable recipients of bystander stress. The level of oxidative stress experienced by melanocytes within the epidermis may thus be profound considering every melanocyte is embedded within a matrix of B36 keratinocytes (Seiberg 2001) and deeper fibroblasts.

Study also disclosed that stress from direct UVA exposure might be reduced in melanocytes because of melanin (Redmond et al. 2014). There is a fresh report that hypopigmented melanocytes from the slaty mouse (Dct mutation) show sharp oxidative sensitivity to UVA irradiation (Wan et al. 2009), although melan-c2J melanocytes are completely devoid of both eumelanin and pheomelanin due to a homozygous Tyr mutation (Bennett et al. 1989). Previous study also presented that UVA convinced more membrane permeability and lipid peroxidation in unpigmented melanocytes (i.e., melan-c) compared to pigmented ones (i.e., melan-a) and more ROS in fibroblasts compared with melanocytes (Kvam and Dahle 2003).

UVA radiation has been established to play a vital job in increasing melanogenesis, via initiation of oxidative stress and weakening of antioxidant defense in melanocytes (Baldea et al. 2009; Panich et al. 2012); development of antioxidant defense system to manage with the overwhelmed oxidative stress could thus be one of the effective and safe approaches to constrain melanogenesis and photoinduced skin damage.

UV irradiation along with alpha-melanocyte-stimulating hormone can trigger an immediate stimulation of tyrosinase activity and increase biosynthesis of melanin through transcriptional and translational upregulation of tyrosinase probably through ROS-mediated pathways (Legros et al. 1981; Peng et al. 2014; Zi et al. 2009). Findings showed that UVA irradiation was capable to incite melanin production and activity and protein level of tyrosinase in association with oxidative stress, indicated by enhanced formation of ROS and 8-OHdG as well as GSH depletion.

The antioxidant and UVA filter might potentially provide early protection against UVA-induced oxidative stress in correlation with enhanced melanogenesis, probably through indirect regulation of Nrf2-ARE pathway. Moreover, the study showed quercetin (a dietary phenolics), to yield the inhibitory effects at lower doses, indicating that quercetin a powerful antioxidant having solid UVA absorption property may produce the greatest protective effects on UVA-induced melanogenesis, oxidative damage and downregulation of Nrf2 and its downstream antioxidants. Hence, abilities to reverse impaired Nrf2 signaling pathway are probably associated with antioxidant capacities of photoprotective agents (Chaiprasongsuk et al. 2016).

Oxidative stress encouraged by ultraviolet A (UVA) radiation has been acknowledged to play a decisive role in physiological and biological stress responses including dysregulation of melanogenesis in melanocytes and/or melanoma cells (Panich et al. 2011). Though melanin production predominantly controlled by tyrosinase shows a constructive role in protecting the skin against detrimental effects of UV radiation.

Although melanin showed photoprotective properties, study revealed that excessive formation of melanin could be harmful, in particular following UV exposure (Swalwell et al. 2012). The role of UVA radiation has been verified in increasing melanogenesis somewhat through induction of oxidative stress and impairment of antioxidant defense in melanoma cells (Slocum and Kensler 2011); improvement of antioxidant defense system to cope with the overwhelmed oxidative stress could thus be one of the effective and safe approaches to inhibit melanogenesis and photodamaged skin. Nrf2- nuclear factor and significant transcription factor are

governing the antioxidant response in various tissues including the skin. The skin has been reported to play a beneficial role in cellular function and integrity by shielding skin cells plus melanocytes against oxidative insults predominantly from UV radiation (López-Camarillo et al. 2012). The author has made attempts to foster efficient photoprotective agents targeting Nrf2. Highly pigmented, dark skin is more defiant to the injurious effects of environmental UV exposure than light-colored human skin, although the extent to which tanning shields skin from harmful effects including initiation of skin cancer is not acknowledged. Previous work has also investigated whether the skin pigment (melanin) sensitizes or protects isolated DNA or nuclear DNA in melanoma cells from the induction of the premutagenic oxidative damage of DNA, by UVA exposure. Synthetic eumelanin sensitized isolated DNA to induction of the oxidative damage of DNA by UVA, but it also induced the oxidative damage of DNA base in the dark. To know the role of natural melanin of mammalian melanoma cells in the initiation of oxidative damage of DNA base, synthesis of melanin was modulated 5–7-fold in the GLL19 (human melanoma cells) and IGR1 (contain both pheomelanin and eumelanin) in the mouse melanoma cells B16 (contain mainly eumelanin).

Psoralens and ultraviolet A radiation is a potent inducer of melanogenesis in normal human skin. Oral administration of psoralen followed by sunlight UVA radiation (PUVA) enhances human skin pigmentation (Fitzpatrick 1989). This PUVA-induced epidermal melanin pigmentation is a more effective filter than a tan induced by UVB (Gschnait et al. 1978). In addition, PUVA induces repigmentation of the hypomelanotic lesions of vitiligo (Albert et al. 1982), by stimulating proliferation and or migration of follicular melanocytes (Ortonllc et al. 1983). The effect of PUVA on the enzymes regulating melanogenesis is not understood. Burchill et al. (1990) showed that the amount of tyrosinase, the rate-limiting enzyme of melanogenesis, is increased in human skin biopsies after several weeks of PUVA therapy. Tyrosinase, the first enzyme described to regulate melanogenesis, initiates melanogenesis by catalyzing the conversion of tyrosine to L-3, 4-dihydroxyphenylalanine (DOP A) and then to dopaquinone. Synthesized as a precursor form of 55 kDa, tyrosinase undergoes extensive posttranslational modifications in the Golgi apparatus where, after glycosylation, it is found as a mature form of about 70 kDa (Hearing et al. 1978).

A significant UVA dose-dependent increase in tyrosinase activity and melanin synthesis occurs after 6 days of PUVA treatment. Compared to S91, melanocytes require higher doses of UVA irradiation to stimulate melanogenesis and the increase in their tyrosinase activity remains proportionally lower. UVB exposure may cause a similar refractoriness of melanocytes (Friedmann and Gilchrcst 1987) that enhance basal tyrosinase activity in melanocytes, which resulting in reduced effective to PUVA than S91 cells.

UVA-induced single-strand break in human melanocytes was measured in human melanocytes varying only in the amount of pigment produced by culturing at different concentrations of L-tyrosine, low (0.01 mM) and high (0.2 mM), the main precursor of melanin. Pheo and total melanin contents of the cells were also examined through two melanocyte cultures derived from a skin type I and a skin type VI

individual. The correlation study between UVA-induced genotoxicity and pheo and total melanin content showed that the skin type VI melanocytes contained ten times more total melanin and about seven times more pheomelanin than the skin type I melanocytes. The study further revealed that UVA sensitivity is increasing with melanin concentration of melanocytes. Increase of tyrosine level in the culture medium ensured the rise of both pheo and total melanin levels in melanocyte cultures and the melanin composition of skin type I melanocytes became more pheomelanogenic than type VI, although type VI melanocytes remained the same. Study further revealed the skin type VI melanocytes cultured in basic medium demonstrated a very high sensitivity to UVA exposure, which may be due to their high pheo and total melanin content. Initially, the sensitivity of UVA toward melanocytes increases with increasing melanin concentration but unaffected at very high tyrosine concentration. UVA sensitivity did not change after raising their melanin content by culturing at high tyrosine level. Although this result is not applicable for type 1 skin, the skin type I melanocytes proven sensitivity toward UVA when cultured in basic medium, but by increasing their melanin content increase in their UVA sensitivity reported. Thus, UVA-irradiated cultured human melanocytes are photosensitized by their own synthesized chromophores, probably pheomelanin, or by melanin intermediates (Wenczl et al. 1998).

Finally come to the conclusion that enhanced melanin synthesis did not protect against UVA-induced damage of DNA bases. Instead, human melanocytes favor two times more 8-hydroxydeoxyguanosine by exposure of UVA than cells with lower melanin content. Moreover, pre-irradiation of the human melanoma cells, which content both eumelanin and pheomelanin (IGR1) with UVA for 4-h exposure earlier a second exposure of UVA, produced an altered quantity of stimulated 8-hydroxy-deoxyguanosine reliant on the melanin concentration of the cells. Thus, UVA-induced initiation premutagenic oxidative damage of DNA base is due to stimulation of melanin synthesis but not melanin itself (Kvam and Tyrrell 1999).

The study also reveals the key role of peroxynitrite in the excitation of an electron of pigmented melanin to triplet state and thus favors dark CPD formation. When DNA base thymine or cytosine absorbs UV photons, a mutation in melanoma arises as an immediate effect and resulted in the formation of cyclobutane pyrimidine dimers (CPDs). The concept of dark CPDs has arisen after prolonged exposure of melanocytes with UVA. These “dark CPDs” are predominant in melanocytes with pheomelanin (responsible for red hair) of human and murine melanocytes as well as in mouse skin. Further mechanism revealed that formation of dark CPDs arises by peroxynitrite, a combination of reactive oxygen and nitric species, which is initiated by UV radiation and having the capacity to excite an electron to the triplet state. Thus, it coined the term “chemiexcitation” which is responsible for bioluminescence in lower organisms. Excitation of electron in the fragments of pigmented melanin, generating a triplet state energy level, possesses the energy of a UV photon and, finally, initiates DNA photoproduct (CPDs) via energy transfer to DNA. Comparatively less energetic UVA and peroxynitrite play a key role in permeabilization of the nuclear membrane, therefore permitting entrance to melanin. Thus, it proved melanin an evidently carcinogenic as well as protective property.

The study also revealed that chemiexcitation activates pathogenesis in internal tissues since the identical chemistry ought to occur wherever near melanocytes triggered by superoxide and nitric oxide (Brash 2016). In the biological system, excited states are characteristically touched, when two pyrimidines (thymine or cytosine) present simultaneously and are exposed to UV photon, which results in excitation of an electron to produce CPDs. Thus, such DNA photoproduct is a source of mutation by linking two adjoining bases of DNA and, finally, distorted the DNA double helix, controlling alterations or favoring cell death mechanism. In lower organism the bioluminescence ATP can stimulate electrons chemically "chemiexcitation" and excited-state energy to releases to visible light. Study further reported that numerous chemical and enzymatic reactions are found to be capable of chemiexcitation without ATP. The nevertheless conflicting result has been found in case of chemiexcitation in mammals. Freshly reported that chemiexcitation really happens in the melanocytes (responsible for hair and skin color) of mammalian. It is trailed by the biophysical transmission of the energy to DNA and helps in the formation of CPDs and showed interconnected to melanoma growth (Premi et al. 2015).

References

Albert, D. M., Todes-Taylor, N., Wagoner, M., Nordlund, J. J., & Lerner, A. B. (1982 Jan). Vitiligo or halo nevi occurring in two patients with choroidal melanoma. *Archives of Dermatology, 118*(1), 34–36.

Baldea, I., Mocan, T., & Cosgarea, R. (2009). The role of ultraviolet radiation and tyrosine stimulated melanogenesis in the induction of oxidative stress alterations in fair skin melanocytes. *Experimental Oncology, 31*, 200–208.

Bennett, D. C., Cooper, P. J., Dexter, T. J., et al. (1989). Cloned mouse melanocyte lines carrying the germline mutations albino and brown: Complementation in culture. *Development, 105*, 379–385.

Brash, D. E. (2016, June). UV-induced melanin chemiexcitation: A new mode of melanoma pathogenesis. *Toxicologic Pathology, 44*(4), 552–554.

Burchill, S. A., Marks, J. M., & Thody, A. J. (1990). Tyrosinase synthesis in diRerent skin types and the clfccts of a-mclanocytc-stimulatillg hormonc and cyclic AMP *fll" est* DCI'II/fllol*, 95,* 558–561.

Chaiprasongsuk, A., Onkoksoong, T., Pluemsamran, T., & Limsaengurai, S. (2016). Uraiwan Panich. Photoprotection by dietary phenolics against melanogenesis induced by UVA through Nrf2-dependent antioxidant responses. *Redox Biology, 8*, 79–90.

Fitzpatrick, T. B., Forlot, P., Pathak, M. A., & Urbach, F. (1989). *Psoralens: Past, present, and future of Photochemoprotection and other biological activities* (pp. 5–10). Paris: John Libbey Eurotext.

Friedmann, P. S., & Gilchrcst, B. (1987). Ultr:lviolet radi:ltion directly induccs pigmcllt production b)' cultured hUlllan melanocytes.) *Cdl PIt), si, 1 133*, 88–94

Gschnait, F., Brcnner, W., & Wolff', K. (1978). Photoprotective CUCCI'of" psor:1lcn-UVA-induced tan. *Arclt Dermnlol* Re.•*, 263*, 181–188

Haywood, J., Francis, P., Dubovik, O., Glew, M., & Holben, B. (2003). Comparison of aerosol size distributions, radiative properties, and optical depths determined by aircraft observations and sun photometers during SAFARI 2000. *Journal of Geophysical Research, 108*(D13), 8471. https://doi.org/10.1029/2002JD002250.

Hearing, V. J., Nicholson, J. M., Montague, P. M., Ekel, T. M., & Tomecki, K. J. (1978). Mammalian tyrosin:1sc structural and functional interelationship of isocllzy.-nes. *Di(Jritilll/3ioph), s Ac, n, 522*, 327–339.

Jhappan, C., Noonan, F., & Merlino, G. (2003). UV radiation and cutaneous malignant melanoma. *Oncogene, 22*, 3099–3112. http://dx.doi.org/10.1038/sj.onc.1206450

Kvam, E., & Dahle, J. (2003). Pigmented melanocytes are protected against ultraviolet-A-induced membrane damage. *The Journal of Investigative Dermatology, 121*, 564–569.

Kvam, E., & Tyrrell, R. M. (1999, August). The role of melanin in the induction of oxidative DNA base damage by ultraviolet A irradiation of DNA or melanoma cells. *The Society for Investigative Dermatology, 113*(2), 209–213.

Legros, F., Coel, J., Doyen, A., Hanson, P., Van Tieghem, N., Vercammen- Grandjean, A., Fruhling, J., & Lejeune, F. J. (1981). Alpha-Melanocyte-stimulating hormone binding and biological activity in a human melanoma cell line. *Cancer Research, 41*, 1539–1544.

López-Camarillo, C., Ocampo, E. A., Casamichana, M. L., Pérez-Plasencia, C., Alvarez-Sánchez, E., & Marchat, L. A. (2012). Protein kinases and transcription factors activation in response to UV-radiation of skin: Implications for carcinogenesis. *International Journal of Molecular Sciences, 13*, 142–172.

Mouret, S., Baudouin, C., Charveron, M., Favier, A., Cadet, J., & Douki, T. (Sep 2006). Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. *Proceedings of the National Academy of Sciences, 103*(37), 13765–13770.

Ortonllc, J. P., Moshcr, D. B., & Fitzpatrick, T. B. (1983). *llllld OtJ,er H)'IWllldmlOsis of Hair alld Skill.* Plenull1 Press, New York, PI' 257–258. *Journal of Investigative Dermatology (2014) 134*, 1083–1090; https://doi.org/10.1038/jid.2013.479; published online 12 December 2013.

Panich, U., Tangsupa-a-nan, V., Onkoksoong, T., Kongtaphan, K., Kasetsinsombat, K., Akarasereenont, P., & Wongkajornsilp, A. (2011). Inhibition of UVA- mediated melanogenesis by ascorbic acid through modulation of antioxidant defense and nitric oxide system. *Archives of Pharmacal Research, 34*, 811–820.

Panich, U., Onkoksoong, T., Limsaengurai, S., Akarasereenont, P., & Wongkajornsilp, A. (2012). UVA-induced melanogenesis and modulation of glutathione redox system in different melanoma cell lines: the protective effect of gallic acid. *The Journal of Photochemistry and Photobiology B, 108*, 16–22.

Peng, H. Y., Lin, C. C., Wang, H. Y., Shih, Y., & Chou, S. T. (2014). The melanogenesis alteration effects of Achillea millefolium L. essential oil and linalyl acetate: Involvement of oxidative stress and the JNK and ERK signaling pathways in melanoma cells. *International Journal of Molecular Sciences, 11*, 1082–1089.

Premi, S., Wallisch, S., Mano, C. M., Weiner, A. B., Bacchiocchi, A., Wakamatsu, K., Bechara, E. J., Halaban, R., Douki, T., & Brash, D. E. (2015). Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure. *Science, 347*, 842–847. [PubMed: 25700512].

Redmond, R. W., Rajadurai, A., Udayakumar, D., Sviderskaya, E. V., & Tsao, H. (2014). Melanocytes are selectively vulnerable to UVA-mediated bystander oxidative signaling. *Journal of Investigative Dermatology, 134*(4), 1083–1090.

Seiberg, M. (2001). Keratinocyte–melanocyte interactions during melanosome transfer. *Pigment Cell Research, 14*, 236–242.

Slocum, S. L., & Kensler, T. W. (2011). Nrf2: Control of sensitivity to carcinogens. *Archives of Toxicology, 85*, 273–284.

Swalwell, H., Latimer, J., Haywood, R. M., & Birch-Machin, M. A. (2012). Investigating the role of melanin in UVA/UVB- and hydrogen peroxide-induced cellular and mitochondrial ROS production and mitochondrial DNA damage in human melanoma cells. *Free Radical Biology & Medicine, 52*, 626–634.

Turro, N. J., Ramamurthy, V., & Scaiano, J. C. (2010). *Modern molecular photochemistry of organic molecules*. Sausalito: University Science Books.

Valencia, A., & Kochevar, I. E. (2008). Nox1-based NADPH oxidase is the major source of UVA-induced reactive oxygen species in human keratinocytes. *The Journal of Investigative Dermatology, 128*, 214–222.

Wan, J., Liu, X. M., Lei, T. C., et al. (2009). Effects of mutation in dopachrome tautomerase on melanosome maturation and anti-oxidative potential in cultured melanocytes. *Zhonghua Yi Xue Za Zhi, 89*, 1707–1710.

Wenczl, E., Van der Schans, G. P., Roza, L., Kolb, R. M., Timmerman, A. J., Smit, N. P. M., Pavel, S., & Schothorst, A. A. (1998). (Pheo)melanin photosensitizes UVA-induced DNA damage in cultured human melanocytes. *The Society for Investigative Dermatology, 111*(4), 678–682.

Zi, S. X., Ma, H. J., Li, Y., Liu, W., Yang, Q. Q., Zhao, G., & Lian, S. (2009). Oligomeric proanthocyanidins from grape seeds effectively inhibit ultraviolet-induced melanogenesis of human melanocytes in vitro. *International Journal of Molecular Medicine, 23*, 197–204.

Future Prospective of Nanotechnology in Skin Cancer Therapeutics

Lipika Ray

Abstract

Melanoma is notorious and very aggressive skin cancer which is well known for its multidrug resistance. Thus, patients having melanoma have a low survival rate. Traditional anticancer treatment for melanoma with dacarbazine, interleukin-2 and interferon-alpha-2b, etc., showed low response and survival rates. However, recently targeted nanoparticle-based therapy displayed good response with better survival rates in melanoma-bearing patients. Better survival rate with very low adverse effects has been observed with targeted nanomedicines in animal models. Thus, numerous nanomedicines are currently under clinical trials worldwide.

Keywords

Nanoparticles · Metastasis melanoma · Melanoma · Targeting · Skin cancer

1 Introduction

Skin cancer is mostly prevalent in Caucasian race and one of the aggressive cancers among all types of cancers (D'Orazio et al. 2013). Skin cancers are mainly two types depending on their originate area and clinical behavior: a) nonmelanocytic skin cancers (NMSC) and b) malignant melanoma (MM) (Simões et al. 2015). NMSC is again two types: basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Melanoma is one of the most notorious and aggressive skin cancers and generates from melanocytes. Multidrug resistance (MDR) is very common in the case of melanomas. In 2014, ~ 77 thousand new cases of melanoma were

L. Ray (✉)
Pharmaceutics and Pharmacokinetics Division, CSIR-Central Drug Research Institute, Lucknow, Uttar Pradesh, India

A. Dwivedi et al. (eds.), *Skin Aging & Cancer*,
https://doi.org/10.1007/978-981-13-2541-0_9

diagnosed in the United States (http://www.cancer.gov/cancertopics/pdq/treatment/melanoma/patient). According to National Cancer Institute of America (NCIA), melanoma is seventh and fifth diagnosed malignancies among women and men, respectively (http://www.cancer.gov/researchandfunding/snapshots/melanoma).

2 Skin Cancer

2.1 Nonmelanocytic Skin Cancer (NMSC)

2.1.1 Basal Cell Carcinoma (BCC)

Worldwide, nearly 3 million new cases of NMSCs are reported in the USA only (Medical Center University of California 2007). The important etiological factors behind NMSCs are UV light, chemical carcinogens, and ionizing radiation. Among all NMSCs, 80–85% is due to BCC and most common type of skin cancer. Superficial BCC looks like a subacute or chronic dermatitis with red, slightly wrinkled, and scaled appearance on the most exposed areas (Goldberg 1996). The shape can be oval or round with a poorly defined border and the center can be fibrotic.

2.1.2 Squamous Cell Carcinoma (SCC)

On the other hand, 15–20% of NMSCs include SCC where the growth shows local destructiveness and invades in surrounding tissue and, thus, causes more death than BCC (Narayanan et al. 2010a). Occurrence of SCC is gradually increasing; however the rate depends on geographic position (Lomas and Bath-Hextall 2012). The factors behind SCC development can be categorized into extrinsic (UVA, human papilloma virus (HPV), ionizing radiation, and chemical substances) and intrinsic (genodermatoses, preexisting skin lesion, immunosuppression, and preexisting actinic keratosis).

2.2 Melanoma

Malignant melanoma (MM) develops from the transformation of melanocytes which produce melanin, skin pigment. The melanoma is very much prevalent among light-skinned population and is excessively exposed to sun radiation. The melanoma-affected countries are Australia, Europe, United States, Slovenia, etc. The diagnosed melanoma patients per 100,000 people are 50, 10, 20, and 23 for Australia, Europe, United States, and Slovenia, respectively. It has been found that occurrence of malignant melanoma among females is more than males in Slovenia (Slora 2016). However, MM is more frequent in males than in females worldwide. According to 2010 report, the risk factor for developing MM of Caucasian population is 16 times more than in Afro-Americans and 10 times greater than in Latin-Americans (Narayanan et al. 2010b). The most important etiological issues are UV light, constitutional factors (race, pigmentation, and genetic predisposition), and other factors (occupation, smoking, diet, endometriosis, oral contraceptives, TNF inhibitors, etc.) (Sachdeva 2009).

2.2.1 The Main Factors for the Development of MM

Skin Type

The risk of MM depends on the skin type. Light skinned are in the highest risk, i.e. 1 person out of 40, while Latin-Americans are in the lower risk, i.e. 1 person out of 200, and dark-skinned people, having more melanin, are in the lowest risk, i.e. 1 in 1000. Figure 1 depicts the different Caucasian skin types and among them types 1 and 2 are in the highest risk. Acral lentiginous melanoma, a type of skin melanoma, usually arises in dark-skinned population, which is mostly observed on palms, soles of feet, in the oral mucosa, and under the nails, less exposed to sunlight.

Sun Radiation

The risk of NMSC and MM depends on both UV exposure and skin type as both UVA and UVB, natural or artificial, are cancerogenic. Although UV radiation is desired in vitamin D generation, strong and long exposure of UV radiation damages genes and suppresses immune system (Simões et al. 2015). Artificial tanning is banned as it is carcinogenic.

Nevi

The atypical nevi also contribute as the risk factor of MM development along with UV exposure and skin type. Generally, nevi, uneven color and shape and larger than 6 mm diameter, are the risk factor for MM development. Atypical nevi syndrome is called where a person is having >50 atypical nevi. The presence of several atypical nevi increases MM risk ~fivefold. Therefore, removal of atypical nevi is not advisable (Simões et al. 2015).

Age

Usually, the chance of MM development increases with age (approximately 62 years); however, teens and adults are also susceptible toward MM (Slora 2016).

Gender

Worldwide, men are more at risk (1.5 times) toward MM than women; however, Slovenians are the exception where the female is in higher risk than the male (Slora 2016).

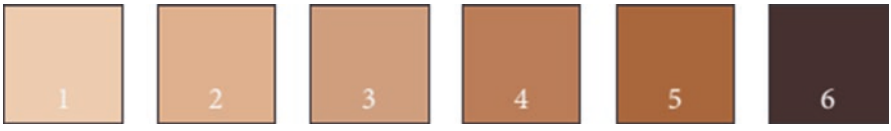

Fig. 1 According to Fitzpatrick and Sober (Fitzpatrick and Sober 1985), skin type (1) rarely tans and always reddens; type (2) frequently reddens and tans rarely; type (3) slightly reddens and tans gradually; type (4) reddens rarely and tans easily; type (5) reddens rarely and tans easily; type (6) never reddens

Immunosuppression

Immunosuppression is also another criterion behind MM development. Immunosuppressed individual is higher risk to MM (Onkolõski Iñstitut Ljubljana, Rak v Sloveniji 2012).

Previously Removed

Formerly removed MM patients are 3–7% higher risk for new MM development (Onkolõski Iñstitut Ljubljana, Rak v Sloveniji 2012).

Family History

Family members are ~5–10% in higher risk for the development of MM when one or two persons in the same family are MM affected. Mostly, BRAF (50% mutation), NRAS (~30%), KIT (~1%), p53 (~5%), PTEN (~50%), MC1R, CDK4, CDKN2A, POT1, TERT, and BAP1 genes are found mutated in the case of MM development.

If color, shape, or size of the nevus changes or it bleeds/itches, or pains, nevus has the potential to develop MM. For long-standing nevus, ABCDE (asymmetry, border, color, dimension, and evolution) (Califano and Nance 2009) criteria should be noticed closely.

Current therapy includes surgery followed by chemotherapy; however, recurrence and multidrug resistance are the challenges. Fortunately, nanotechnology development played a pivotal role in chemotherapy and targeted chemotherapy. Small-size nanoparticles show enhanced permeability and retention (EPR) effect and stay longer time inside systemic circulation. Thus, the uptake of drug-loaded nanoparticles is higher than bulk drug.

Nanoparticle (NP)-Based Drug Delivery to Melanoma

Nanoparticle-based drug delivery to melanoma permits therapeutic drugs to accumulate specifically in tumor sites without leading severe adverse effects. NP-based drug delivery to melanoma can be three kinds: passive targeting, active targeting, and triggered drug delivery responsive to different internal/external stimuli. Passive targeting occurs through EPR effect, where the tumor vasculature is leaky in nature, and NP-based drug can be accumulated in the target area (Matsumura and Maeda 1986). On the contrary, NP-based drug interacts with tumor cell surface receptors and accumulates inside the tumor cells specifically in active targeting in active targeting. Basically, in the case of active targeting, NPs are modified with targeting ligands, such as antibodies, peptides, nucleic acid-based ligands, or small molecules, which bind to receptors overexpressed on the tumor cell surface (Yu et al. 2010). Therapeutic drug delivery through active targeting is more specific to target tissues than passive targeting. By blending bio-responsive NPs with internal or external stimuli (such as hyperthermia, pH gradient, light, alternating magnetic field and acoustic), drug release can be successfully attained. These types of NPs are intended to only release the melanoma-specific drugs upon applying internal or external stimuli, therefore maximizing drug release at the specific sites of tumors.

Passive Targeting to Melanoma Cells

Docetaxel (DTX) was attached to carboxymethyl chitosan (CMCS) by a biodegradable linker to obtain CMCS-DTX conjugates which displayed enhanced antitumor effect than DTX alone which prolonged survival time of B16 melanoma-bearing mice significantly (Liu et al. 2013). According to Zhang et al. (Zhang et al. 2011), PTX loaded in Pluronic P123 and F127 block copolymer nanoparticle (PF-PTX) significantly inhibited tumor growth and prolonged the survival time in pulmonary and subcutaneous B16-F10 melanoma model than Taxol. DOX-loaded monomethoxy poly(ethylene glycol)-poly(epsilon-caprolactone) (DOX/MPEG-PCL) micelles showed increased cytotoxicity of DOX in B16 melanoma cells. DOX/MPEG-PCL NP showed significantly improved antitumor activity with less adverse effects in melanoma xenograft model compared to free DOX (Zheng et al. 2011). NP also has been used to deliver siRNA and DNA to melanoma cells using chitosan. Yang et al. (Yang et al. 2011) delivered VEGF-siRNA successfully to melanoma cells by using chitosan (CTS/siRNA) NPs and CTS/siRNA NPs showed significant transfection efficacy with enhanced VEGF gene silencing efficiency in B16-F10 melanoma cells with no cytotoxicity. A cationic micelle NP, PEG-poly(propylene sulfide)-poly(ethylene imine) (PEG-PPS-PEI), delivered plasmid DNA (pDNA) to melanoma cells which displayed enhanced transfection efficiency compared to naked pDNA with less cytotoxicity (Velluto et al. 2011). Liposome-based nanocarriers have also been used for passive targeting drug delivery to melanoma. Both cytochalasin D and N,N,N-trimethylphytosphingosine-iodide (TMP-I) are anticancer drugs for melanoma but severe toxic effects limit their medical use in clinic. However, nanoencapsulation of cytochalasin D efficiently inhibited tumor growth and prolonged melanoma-bearing mice survival (Huang et al. 2012). TMP-I nanoparticles not only inhibited melanoma tumor growth but reduced angiogenesis also (Song et al. 2012). Nanoformulation of melittin, venom of honeybee (Apis mellifera), showed significant reduction of tumor growth in murine melanoma model with apparent no toxicity (Soman et al. 2008a, b, 2009). Huang et al. (2013) also reported that α-melittin-based lipid NP (α-melittin-NP) displayed safer dose, reduced tumor growth in melanoma-bearing mice, compared to free melittin.

Active Targeting to Melanoma Cells

The effective targeted therapy over passive targeting is the use of antibodies (biotherapeutics) which binds to the receptor molecules overexpressed on the melanoma cell surface. BIND-014 is one of the antibodies which has been used to selectively bind to melanoma receptors. Again, Ep1, monoclonal antibody, was conjugated with cisplatin and encapsulated inside ferritin cage (HFt–Pt–Ep1). Since Ep1 is the antibody of CSPG4, HFt–Pt–Ep1 NP selectively binds with CSPG4 (+) melanoma cell line but not to CSPG (−) breast carcinoma cell line. The human melanoma-specific antigen CSPG4 was conjugated to a single cisplatin-encapsulated ferritin cage (HFt–Pt–Ep1). HFt–Pt–Ep1 demonstrated specific binding to a CSPG4 (+) melanoma cell line, but not to a CSPG (−) breast carcinoma cell line (Falvo

et al. 2013). Melanocortin receptor-1 (MCR-1), laminin receptor, fibroblast growth factor receptor (FGFR), sigma receptor and somatostatin receptor (SSTR), etc., are common receptors that can be used for active targeting of melanoma treatment. Truncated FGF was loaded inside PEGylated liposomes and PTX/DOX co-encapsulated and used for active targeting which showed enhanced accumulation in melanoma tissues but less concentration in other organs such as the heart, kidney, and lung compared with nontargeted liposomes or free PTX and DOX (Cai et al. 2012; Chen et al. 2010a). Octreotide is known to be overexpressed on the melanoma cell surface. DOX encapsulated octreotide-PEG-phosphatidylethanolamine (Oct-PEG-PE)-based liposomal NP displayed a large amount of DOX accumulation inside the melanoma cells (Sun et al. 2010). Again, 5-fluorouracil (5-FU)-loaded Tyr-Ile-Gly-Ser-Arg (YIGSR) peptide-based PEGylated nanospheres target specifically laminin receptors and showed reduction in tumor growth in B16-F10 melanoma mice model compared to nontargeted liposomes and free 5-FU (Dubey et al. 2010). Etoposide also targets laminin and etoposide-loaded NP significantly targets melanoma tissues (Ukawala et al. 2012). The first Tf receptor-targeted NP is CALAA-01 for siRNA delivery and showed enhanced antitumor effect in melanoma model. c-Myc or c-Myc/MDM2/VEGF siRNA delivery done by sigma receptor is targeted by N,N-distearyl-N-methyl-N-2-(N′-arginyl) aminoethyl ammonium chloride (DSAA) NPs and displayed increased antitumor activity in melanoma mice model (Chen et al. 2010b; Yang et al. 2012). Interestingly, MCR-1 receptor-targeted NPs loaded with thymidine kinase gene (HSVtk) showed significant melanoma growth inhibition compared to nontargeted polyplexes (Durymanov et al. 2012). Although actively targeted NPs are promising in clinical trial phases I and II, till now there are no actively targeted NPs available in the market.

Integrin Targeting

Usually, a wide range of integrins like αvβ3, αvβ5, α5β1, etc., are overexpressed on melanoma cell surface. Integrins are important in tumor growth, invasion, angiogenesis, and metastasis. Therefore, finding good target is important for the treatment of melanoma by active targeting (Bergers and Benjamin 2003; Desgrosellier and Cheresh 2010). Arginylglycylaspartic acid (RGD), a peptide, is responsible for cell adhesion and has high affinity toward integrin αvβ3. RGD peptide is very popular in the case of integrin targeting. Cyclic RGD peptides were modified with PEGliposomes to target melanoma actively. Antitumor drug (DOX) and antivascular agent combretastatin A4 (CA4) were encapsulated inside the cyclic RGDyK NP and showed enhanced antitumor and antiproliferative effect in melanoma (B16-F10) cells compared to nontargeted and free drug-treated groups (Wang et al. 2011a). According to Wang et al. PTX and CA4 encapsulated cRGDfK peptide and displayed significant inhibition of tumor growth (Wang et al. 2011b). Again, integrin α5β1-targeted DOX-loaded liposomal NP displayed enhanced antitumor activity in B16-F10 melanoma-bearing mice than with nontargeted and free drug groups (Dai et al. 2012). It is evident that dual targeting NPs are more significant toward tumor inhibition than mono-targeting. Both integrins αvβ3 and α5β1 were targeted by 12-amino acid synthetic peptide C16Y which showed enhanced antitumor

activity in B16 melanoma-bearing mice. However, antitumor activity of dual targeting peptide C16Y was significantly higher in case of liposomal C16Y NPs in melanoma mice (Hamano et al. 2012). Similar observation was obtained when galectin-1-specific anginex [Anx] and αvβ3 integrin-specific RGD were attached to liposomes and displayed significantly synergistic antitumor activity in B16-F10 melanoma-bearing mice than Anx and RGD-targeted liposomes (Kluza et al. 2012). Recently, DTX-conjugated TH10 peptide (TAASGVRSMH) NPs exhibited targeting ability toward NG2 which is a proteoglycan receptor overexpressed in B16F10-luc-G5 melanoma mice model (Guan et al. 2014). Although many exciting clinical observation was obtained in the case of mono-/dual-targeted therapy, application in clinic is yet to achieve.

Stimuli-Responsive Targeted Delivery

Tumor tissues are very alike to normal tissues; however there are few characteristics present in tumor which can be activated through stimuli. For example, tumor tissues are more acidic in nature, more heat sensitive than normal tissues. Thus, this stimuli-guided NP-loaded drug delivery would be beneficial. The tumor-specific stimuli are pH, temperature, and light.

pH Value

The selection of polymer is very important in the case of pH-dependent NP-based drug delivery. Usually, microenvironment of tumor is acidic and this low pH may trigger the breaking of the acid-sensitive bonds (i.e., ester, hydrazone, etc.) present in polymer-based NPs. Low pH-guided NP shell destruction leads to significant and targeted drug release inside the tumor tissue. Zhu et al. synthesized long hydrophobic chain attached (C18) gemcitabine, a drug for melanoma treatment, micelles (PHC) where hydrazone and amide linkage was found to be more sensitive under low pH (tumor environment) than acid-insensitive micelles (Zhu et al. 2012). Again, DOX-loaded micelles with methacrylamide bonding are more susceptible for low pH environment and undergo hydrolysis followed by drug release inside the tumor tissue. It is well demonstrated in B16-F10 melanoma mice model and displayed enhanced antitumor activity compared to free DOX and MA control (Talelli et al. 2010). PEG-chitosan nanogel also gives pH-responsive drug release under low pH (tumor endosome pH ~5.0) and can be regulated by external effect (cooling or heating). This treatment is called chemo-thermal therapy and is very effective to reduce the growth of melanoma in mice model (Zhou et al. 2013). The potent anticancer drug, like cisplatin, also was attached to thermo-responsive NPs (PNIPAM-Au NPs) which release anticancer drug inside the A875 melanoma cells in acidic pH (Xiao et al. 2013). pH-responsive NPs not only successfully deliver drug to melanoma cell but nucleic acids (DNA or RNA) also. Carboxymethyl poly(L-histidine) (CM-PLH) is a pH-responsive polymer which successfully delivers DNA in tumor-bearing B16-F10 mice model where DNA is protected from degradation by polymer release DNA in the endosomal pH (Gu et al. 2012). In another study, Tao et al. found that A375 melanoma cells are in general hypoxic and it accumulates more lactic acid and makes subcellular environment acidic. The expression of TfR is more in hypoxic

condition of A375 melanoma cells. Thus, combination of receptor-based active targeting and pH-responsive NP-based therapy is effective in A375 melanoma cells and it has been found synergistic antitumor effect in A375 melanoma cells (Tao et al. 2007; Brahimi-Horn et al. 2011).

Temperature

Superparamagnetic iron oxide nanoparticles (SPIONs) generate heat under magnetic field and gold NPs generate heat on the application of shortwave radio frequency which can trigger drug release and kill melanoma cells. Heat shock protein (Hsp) 90 inhibitor reduces Akt repression in B16 melanoma cells and creates hyperthermia and reduces the tumor growth. The combination of ferromagnetic and thermo-responsive behavior (Hsp 90 inhibitor, i.e., geldanamycin) in one NP leads to higher antitumor effect than control (Ito et al. 2009). Another study showed that thermo-responsive liposome loaded with DOX (DOX-TSL) exhibited enhanced antitumor effect compared to DOX alone (Li et al. 2013).

Light

Drug delivery by light-sensitive NPs releases drug inside the melanoma cells upon application of ultraviolet (UV), visible light, or near-infrared (NIR) light. Basically, this type of NPs changes light energy to heat wave and releases drug followed by kill melanoma cells. For example, CdTe and CdSe QDs are capable of converting light to heat easily (Bédard et al. 2010). Silica shell coated with CdTe QDs significantly reduced melanoma growth upon laser irradiation than without laser (Chu et al. 2012). Alpha-melanocyte-modified hollow gold nanospheres also kill melanoma cells specifically after application of NIR radiation in tumor-bearing nude mice model (Lu et al. 2009). Another light-responsive antitumor therapy is called photodynamic therapy (PDT) where light-sensitive compound exposed to light generates radicals which react with oxygen and generate toxic compound inside the melanoma cells. It is a completely invasive treatment and is commonly used in the treatment of skin cancer. Chitosan NPs conjugated with aminolevulinic acid photosensitize upon radiation of visible light and were shown enhanced tumor reduction by PDT (Ferreira et al. 2013). It is important to note that visible light-driven PDT is unable to penetrate large and solid mass of melanoma tissue; however NIR has better penetration power inside the solid tumor than visible light.

Clinical Trials of NPs for the Treatment of Melanoma

Having lots of promising results of NPs in an animal model for the treatment of melanoma however, efficacy and safety of nanoparticles for the treatment of melanoma in clinical trials are of great concern (Li et al. 2015). Thus, there needs to be a thorough clinical trial in animal models. Recent ongoing clinical trials are listed in Table 1.

According to clinicaltrials.gov Web site, ~5% of total clinical trials are clinical trials of nanoformulations for the treatment of melanoma. The safety and efficacy of a wide range of nanomedicine like Abraxane, Taxoprexin (docosahexaenoic acid-paclitaxel; Protarga), Caelyx (PEGylated liposomal doxorubicin; Janssen Pharm),

Table 1 Clinical trials of NPs for the treatment of melanoma

NCT no.	Phase	NP formulation	Result	Collaborators
Chemotherapy				
NCT00864253 (clinicaltrials.gov web site)	3	Nanoparticle albumin-bound paclitaxel (Abraxane, ABI-007)	A trial of ABI-007 versus dacarbazine in previously untreated patients with metastatic MM	University of Arizona
NCT00462423 (clinicaltrials.gov web site)	2	Nanoparticle albumin-bound paclitaxel	Abraxane and Avastin as therapy for patients with MM	Celgene Corporation and Genentech
NCT00404235 (Kottschade et al. 2011)	2	Nanoparticle albumin-bound paclitaxel	Carboplatin and ABI-007 in treating patients with stage IV melanoma that cannot be removed by surgery	North Central Cancer Treatment Group; National Cancer Institute (NCI)
NCT00093119 (Hersh et al. 2010)	2	Nanoparticle albumin-bound paclitaxel	A trial of ABI-007 in previously treated patients with metastatic melanoma	Celgene Corporation
NCT00626405 (Kottschade et al. 2013)	2	Nanoparticle albumin-bound paclitaxel	A trial of temozolomide and bevacizumab (TB) or ABI-007, bevacizumab and carboplatin (ABC) in patients with unresectable stage IV MM	North Central Cancer Treatment group; National Cancer Institute (NCI)
NCT00087776 (Bedikian et al. 2011)	3	Docosahexaenoic acid-paclitaxel (Taxoprexin)	Taxoprexin injection vs. dacarbazine in patients with metastatic MM	Luitpold Pharmaceuticals
NCT00244816 (Homsi et al. 2010)	2	Docosahexaenoic acid-paclitaxel	Study of weekly Taxoprexin injection as treatment of patients with metastatic uveal (choroidal) MM	Luitpold Pharmaceuticals
NCT00249262 (Bedikian et al. 2011)	2	Docosahexaenoic acid-paclitaxel	Study of weekly Taxoprexin injection as 1st line treatment of patients with metastatic non-choroidal melanoma	Luitpold Pharmaceuticals
Immunotherapy (Vaccine)				
NCT00044356 (Bedikian et al. 2010)	2	Allovectin-7 (HLA-B7/beta-2 microglobulin plasmid DNA/lipid complex)	Study of high-dose Allovectin-7 in patients with advanced metastatic MM	Vical

(continued)

Table 1 (continued)

NCT no.	Phase	NP formulation	Result	Collaborators
PEGylated interferon				
NCT00221702 (Grob et al. 2013)	3	Peginterferon-α-2b (PEG-Intron)	Comparing adjuvant treatment with PEG-Intron over 36 months versus reference treatment with intron A (interferon-α-2b) over 18 months in cutaneous melanoma patients AJCC Stage II	University hospital, Bordeaux; Schering-Plough
NCT00006249 (Eggermont et al. 2012)	3	Peginterferon-α-2b	PEG-Intron observation after regional lymph node dissection in AJCC stage III (TxN1-2MO) melanoma patients	European Organisation for the Research and Treatment of Cancer (EORTC)
NCT00623402 (Degen et al. 2013)	2	Peginterferon-α-2b	Combined treatment of sorafenib and peginterferon-α-2b in stage IV metastatic MM	University of Schleswig-Holstein; Dermatologic Cooperative Oncology Group

Marqibo (vincristine sulfate liposome injection; Talon Therapeutics), Allovectin-7 (HLA-B7/beta-2 microglobulin plasmid DNA/lipid complex; Vical), ADI-PEG-20 (PEGylated arginine deiminase; Polaris Group), IMCgp100 (melanoma gp100 peptide fused to an anti-CD3 antibody fragment; Immunocore), CR011-vcMMAE (glembatumumab (CR011) (human IgG2 monoclonal antibody) linked to monomethyl auristatin E (MMAE); CuraGen), hu14.18-IL2 (humanized antidisialoganglioside (GD2) antibody (hu14.18)-IL-2 fusion protein; EMD Serono), ALT-801 (IL-2/T-cell receptor fusion protein targeting human p53 antigen peptide epitope (aa264–272); Altor Bioscience), AS1409 (humanized anti-BC1 antibody-IL-12 fusion protein; Antisoma Research), L19IL2 (anti-ED-B fibronectin domain antibody-IL-2 fusion protein; Philogen S.p.A.), PEG-Intron (peginterferon-α-2b; Schering-Plough), ANG1005 (Angiopep-2 paclitaxel conjugate targeting lipoprotein receptor-related protein 1 (LRP-1); AngioChem), etc., have been tested in clinical trials for the treatment of metastatic melanoma.

3 Conclusion

Numerous nanoparticles have been tested for the treatment of melanoma and metastatic melanoma and it is clearly found that targeted nanomedicines are more effective than nontargeted nanoformulations. However, deep understanding is needed to

know the mechanism of action of nanomedicines in melanoma metastasis and immunological functions for the development of melanoma. A thorough study is needed regarding the better efficacy of targeted nanoparticles and their safety issues in higher animal models.

Acknowledgements LR gratefully acknowledges the fellowship provided by CSIR-Senior Research Associateship (Scientists' Pool Scheme) (IA-27607) and CSIR-CDRI, Lucknow for providing facility and support.

References

Bédard, M. F., De Geest, B. G., Skirtach, A. G., Möhwald, H., & Sukhorukov, G. B. (2010). Polymeric microcapsules with light responsive properties for encapsulation and release. *Advances in Colloid and Interface Science, 158*(1–2), 2–14.

Bedikian, A. Y., Richards, J., Kharkevitch, D., Atkins, M. B., Whitman, E., & Gonzalez, R. (2010). A phase 2 study of high-dose Allovectin-7 in patients with advanced metastatic melanoma. *Melanoma Research, 20*(3), 218–226.

Bedikian, A. Y., DeConti, R. C., Conry, R., Agarwala, S., Papadopoulos, N., Kim, K. B., et al. (2011). Phase 3 study of docosahexaenoic acid–paclitaxel versus dacarbazine in patients with metastatic malignant melanoma. *Annals of Oncology, 22*(4), 787–793.

Bergers, G., & Benjamin, L. E. (2003). Tumorigenesis and the angiogenic switch. *Nature Reviews. Cancer, 3*(6), 401–410.

Brahimi-Horn, M. C., Bellot, G., & Pouysségur, J. (2011). Hypoxia and energetic tumour metabolism. *Current Opinion in Genetics & Development, 21*(1), 67–72.

Cai, L., Wang, X., Wang, W., Qiu, N., Wen, J., Duan, X., et al. (2012). Peptide ligand and PEG-mediated long-circulating liposome targeted to FGFR overexpressing tumor in vivo. *Int J Nanomedicine, 7*, 4499–4510.

Califano, J., & Nance, M. (2009). MalignantMelanoma. *Facial Plastic Surgery Clinics of North America, 17*(3), 337–348.

Chen, X., Wang, X., Wang, Y., Yang, L., Hu, J., Xiao, W., et al. (2010a). Improved tumor-targeting drug delivery and therapeutic efficacy by cationic liposome modified with truncated bFGF peptide. *Journal of Controlled Release, 145*(1), 17–25.

Chen, Y., Bathula, S. R., Yang, Q., & Huang, L. (2010b). Targeted nanoparticles deliver siRNA to melanoma. *The Journal of Investigative Dermatology, 130*(12), 2790–2798.

Chu, M., Pan, X., Zhang, D., Wu, Q., Peng, J., & Hai, W. (2012). The therapeutic efficacy of CdTe and CdSe quantum dots for photothermal cancer therapy. *Biomaterials, 33*(29), 7071–7083.

D'Orazio, J., Jarrett, S., Amaro-Ortiz, A., & Scott, T. (2013). UV radiation and the skin. *International Journal of Molecular Sciences, 14*(6), 12222–12248.

Dai, W., Yang, T., Wang, Y., Wang, X., Wang, J., Zhang, X., et al. (2012). Peptide PHSCNK as an integrin alpha5beta1 antagonist targets stealth liposomes to integrin-overexpressing melanoma. *Nanomedicine, 8*(7), 1152–1161.

Degen, A., Weichenthal, M., Ugurel, S., Trefzer, U., Kilian, K., Garbe, C., et al. (2013). Cutaneous side effects of combined therapy with sorafenib and pegylated interferon alpha-2b in metastatic melanoma (phase II DeCOG trial). *Journal der Deutschen Dermatologischen Gesellschaft, 11*(9), 846–853.

Desgrosellier, J. S., & Cheresh, D. A. (2010). Integrins in cancer: Biological implications and therapeutic opportunities. *Nature Reviews. Cancer, 10*(1), 9–22.

Dubey, P. K., Singodia, D., & Vyas, S. P. (2010). Polymeric nanospheres modified with YIGSR peptide for tumor targeting. *Drug Delivery, 17*(7), 541–551.

Durymanov, M. O., Beletkaia, E. A., Ulasov, A. V., Khramtsov, Y. V., Trusov, G. A., Rodichenko, N. S., et al. (2012). Subcellular trafficking and transfection efficacy of polyethylenimine–

polyethylene glycol polyplex nanoparticles with a ligand to melanocortin receptor-1. *Journal of Controlled Release, 163*(2), 211–219.

Eggermont, A. M., Suciu, S., Testori, A., Santinami, M., Kruit, W. H., Marsden, J., et al. (2012). Long-term results of the randomized phase III trial EORTC 18991 of adjuvant therapy with pegylated interferon alfa-2b versus observation in resected stage III melanoma. *Journal of Clinical Oncology, 30*(31), 3810–3818.

Falvo, E., Tremante, E., Fraioli, R., Leonetti, C., Zamparelli, C., Boffi, A., et al. (2013). Antibody-drug conjugates: Targeting melanoma with cisplatin encapsulated in protein-cage nanoparticles based on human ferritin. *Nanoscale, 5*(24), 12278–12285.

Ferreira, D. M., Saga, Y. Y., Aluicio-Sarduy, E., & Tedesco, A. C. (2013). Chitosan nanoparticles for melanoma cancer treatment by photodynamic therapy and electrochemotherapy using aminolevulinic acid derivatives. *Current Medicinal Chemistry, 20*(14), 1904–1911.

Fitzpatrick, T. B., & Sober, A. J. (1985). Sunlight and skin cancer. *New England Journal of Medicine, 313*(13), 818–820.

Goldberg, L. H. (1996). Basal cell carcinoma. *Lancet, 347*(9002), 663–667.

Grob, J. J., Jouary, T., Dréno, B., Asselineau, J., Gutzmer, R., Hauschild, A., et al. (2013). Adjuvant therapy with pegylated interferon alfa-2b (36 months) versus low-dose interferon alfa-2b (18 months) in melanoma patients without macrometastatic nodes: An open-label, randomised, phase 3 European Association for Dermato-Oncology (EADO) study. *European Journal of Cancer, 49*(1), 166–174.

Gu, J., Wang, X., Jiang, X., Chen, Y., Chen, L., Fang, X., et al. (2012). Self-assembled carboxymethyl poly (L-histidine) coated poly (beta-amino ester)/DNA complexes for gene transfection. *Biomaterials, 33*(2), 644–658.

Guan, Y. Y., Luan, X., Xu, J. R., Liu, Y. R., Lu, Q., Wang, C., et al. (2014). Selective eradication of tumor vascular pericytes by peptide-conjugated nanoparticles for antiangiogenic therapy of melanoma lung metastasis. *Biomaterials, 35*(9), 3060–3070.

Hamano, N., Negishi, Y., Fujisawa, A., Manandhar, M., Sato, H., Katagiri, F., et al. (2012). Modification of the C16Y peptide on nanoparticles is an effective approach to target endothelial and cancer cells via the integrin receptor. *International Journal of Pharmaceutics, 428*(1–2), 114–117.

Hersh, E. M., O'Day, S. J., Ribas, A., Samlowski, W. E., Gordon, M. S., Shechter, D. E., et al. (2010). A phase 2 clinical trial of nab–paclitaxel in previously treated and chemotherapy-naive patients with metastatic melanoma. *Cancer, 116*(1), 155–163.

Homsi, J., Bedikian, A. Y., Papadopoulos, N. E., Kim, K. B., Hwu, W. J., Mahoney, S. L., et al. (2010). Phase 2 open-label study of weekly docosahexaenoic acid–paclitaxel in patients with metastatic uveal melanoma. *Melanoma Research, 20*(6), 507–510.

http://www.cancer.gov/cancertopics/pdq/treatment/melanoma/patient

http://www.cancer.gov/researchandfunding/snapshots/melanoma

Huang, F. Y., Mei, W. L., Li, Y. N., Tan, G. H., Dai, H. F., Guo, J. L., et al. (2012). The antitumour activities induced by pegylated liposomal cytochalasin D in murine models. *European Journal of Cancer, 48*(14), 2260–2269.

Huang, C., Jin, H. L., Qian, Y., Qi, S. H., Luo, H. M., Luo, Q. M., et al. (2013). Hybrid melittin cytolytic peptide-driven ultrasmall lipid nanoparticles block melanoma growth in vivo. *ACS Nano, 7*(7), 5791–5800.

Ito, A., Saito, H., Mitobe, K., Minamiya, Y., Takahashi, N., Maruyama, K., et al. (2009). Inhibition of heat shock protein 90 sensitizes melanoma cells to thermosensitive ferromagnetic particle-mediated hyperthermia with low Curie temperature. *Cancer Science, 100*(3), 558–564.

Kluza, E., Jacobs, I., Hectors, S. J., Mayo, K. H., Griffioen, A. W., Strijkers, G. J., et al. (2012). Dual-targeting of alphavbeta3 and galectin-1 improves the specificity of paramagnetic/fluorescent liposomes to tumor endothelium in vivo. *Journal of Controlled Release, 158*(2), 207–214.

Kottschade, L. A., Suman, V. J., Amatruda, T., III, McWilliams, R. R., Mattar, B. I., Nikcevich, D. A., et al. (2011). A phase II trial of nab–paclitaxel (ABI-007) and carboplatin in patients with unresectable stage IV melanoma: A north central Cancer treatment group study, N057E (1). *Cancer, 117*(8), 1704–1710.

Kottschade, L. A., Suman, V. J., Perez, D. G., McWilliams, R. R., Kaur, J. S., Amatruda, T. T., III, et al. (2013). A randomized phase 2 study of temozolomide and bevacizumab or nab–paclitaxel, carboplatin, and bevacizumab in patients with unresectable stage IV melanoma: A north central Cancer treatment group study, N0775. *Cancer, 119*(3), 586–592.

Li, L., ten Hagen, T. L., Hossann, M., Süss, R., van Rhoon, G. C., Eggermont, A. M., et al. (2013). Mild hyperthermia triggered doxorubicin release from optimized stealth thermosensitive liposomes improves intratumoral drug delivery and efficacy. *Journal of Controlled Release, 168*(2), 142–150.

Li, J., Wang, Y., Liang, R., An, X., Wang, K., Shen, G., Yating, T., Zhu, J., & Tao, J. (2015). Recent advances in targeted nanoparticles drug delivery to melanoma. *Nanomedicine: Nanotechnology, Biology, and Medicine, 11*, 769–794.

Liu, F., Feng, L., Zhang, L., Zhang, X., & Zhang, N. (2013). Synthesis, characterization and antitumor evaluation of CMCS–DTX conjugates as novel delivery platform for docetaxel. *International Journal of Pharmaceutics, 451*(1–2), 41–49.

Lomas, J., Leonardi-Bee, J., & Bath-Hextall, F. (2012). A systematic review of worldwide incidence of nonmelanoma skin cancer. *British Journal of Dermatology, 166*(5), 1069–1080.

Lu, W., Xiong, C., Zhang, G., Huang, Q., Zhang, R., Zhang, J. Z., et al. (2009). Targeted photothermal ablation of murine melanomas with melanocyte-stimulating hormone analog conjugated hollow gold nanospheres. *Clinical Cancer Research, 15*(3), 876–886.

Matsumura, Y., & Maeda, H. (1986). A new concept for macromolecular therapeutics in cancer chemotherapy: Mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Research, 46*(12 Pt 1), 6387–6392.

Medical Center University of California. (2007). *Nonmelanoma skin cancer vs. Melanoma*.

Narayanan, D. L., Saladi, R. N., & Fox, J. L. (2010a). Ultraviolet radiation and skin cancer. *International Journal of Dermatology, 49*(9), 978–986.

Narayanan, D. L., Saladi, R. N., & Fox, J. L. (2010b). Ultraviolet radiation and skin cancer. *International Journal of Dermatology, 49*(9), 978–986.

Onkolõski Iñstitut Ljubljana, Rak v Sloveniji. (2012). Edited by M. ˇZakelj Primic, Onkolõski iñstitut Ljubljana, Epidemiologija in Register Raka, Register Raka Republike Slovenije, Ljubljana, Slovenia, 2015.

Sachdeva, S. (2009). Fitzpatrick skin typing: Applications in dermatology. *Indian Journal of Dermatology, Venereology and Leprology, 75*(1), 93–96.

Simões, M. C. F., Sousa, J. J. S., & Pais, A. A. C. C. (2015). Skin cancer and new treatment perspectives: A review. *Cancer Letters, 357*(1), 8–42.

Slora. (2016). *Maligni melanom (C43): Slora*. http://www.slora.si/c/documentlibrary/getfile?uuid=c2e610c7-5353-40dd-93e9-1b1b2320e3e1&groupId=11561

Soman, N., Marsh, J., Lanza, G., & Wickline, S. (2008a). New mechanisms for nonporative ultrasound stimulation of cargo delivery to cell cytosol with targeted perfluorocarbon nanoparticles. *Nanotechnology, 19*(18), 185102.

Soman, N. R., Lanza, G. M., Heuser, J. M., Schlesinger, P. H., & Wickline, S. A. (2008b). Synthesis and characterization of stable fluorocarbon nanostructures as drug delivery vehicles for cytolytic peptides. *Nano Letters, 8*(4), 1131–1136.

Soman, N. R., Baldwin, S. L., Hu, G., Marsh, J. N., Lanza, G. M., Heuser, J. E., et al. (2009). Molecularly targeted nanocarriers deliver the cytolytic peptide melittin specifically to tumor cells in mice, reducing tumor growth. *The Journal of Clinical Investigation, 119*(9), 2830–2842.

Song, C. K., Lee, J. H., Jahn, A., Choi, M. J., Namgoong, S. K., Hong, S. S., et al. (2012). In vitro and in vivo evaluation of N,N,N-trimethylphytosphingosineiodide (TMP) in liposomes for the treatment of angiogenesis and metastasis. *International Journal of Pharmaceutics, 434*(1–2), 191–198.

Sun, M., Wang, Y., Shen, J., Xiao, Y., Su, Z., & Ping, Q. (2010). Octreotidemodification enhances the delivery and targeting of doxorubicin-loaded liposomes to somatostatin receptors expressing tumor in vitro and in vivo. *Nanotechnology, 21*(47), 475101.

Talelli, M., Iman, M., Varkouhi, A. K., Rijcken, C. J., Schiffelers, R. M., Etrych, T., et al. (2010). Core-crosslinked polymeric micelles with controlled release of covalently entrapped doxorubicin. *Bimaterials, 31*(30), 7797–7804.

Tao, J., Liu, Y. Q., Li, Y., Peng, J. L., Li, L., Liu, J., et al. (2007). Hypoxia: Dual effect on the expression of transferrin receptor in human melanoma A375 cell line. *Experimental Dermatology, 16*(11), 899–904.

Ukawala, M., Chaudhari, K., Rajyaguru, T., Manjappa, A. S., Murthy, R. S., & Gude, R. (2012). Laminin receptor-targeted etoposide loaded polymeric micelles: A novel approach for the effective treatment of tumor metastasis. *Journal of Drug Targeting, 20*(1), 55–66.

Velluto, D., Thomas, S. N., Simeoni, E., Swartz, M. A., & Hubbell, J. A. (2011). PEG-b–PPS-b–PEI micelles and PEG-b–PPS/PEG-b–PPS-b–PEI mixed micelles as non-viral vectors for plasmid DNA: Tumor immunotoxicity in B16F10 melanoma. *Biomaterials, 32*(36), 9839–9847.

Wang, Y., Yang, T., Wang, X., Dai, W., Wang, J., Zhang, X., et al. (2011a). Materializing sequential killing of tumor vasculature and tumor cells via targeted polymeric micelle system. *Journal of Controlled Release, 149*(3), 299–306.

Wang, Z., Chui, W. K., & Ho, P. C. (2011b). Nanoparticulate delivery system targeted to tumor neovasculature for combined anticancer and antiangiogenesis therapy. *Pharmaceutical Research, 28*(3), 585–596.

Xiao, M., Liang, R., Deng, R., Dong, L., Yi, S., Zhu, J., et al. (2013). pH-Sensitive cisplatin-loaded gold nanoparticles for potential melanoma therapy. *Journal of Controlled Release, 172*, e44.

Yang, Y., Liu, X., Zhang, D., Yu, W., Lv, G., Xie, H., et al. (2011). Chitosan/VEGFsIRNA nanoparticle for gene silencing. *Journal of Controlled Release, 152*(Suppl 1), e160–e161.

Yang, Y., Li, J., Liu, F., & Huang, L. (2012). Systemic delivery of siRNA via LCP nanoparticle efficiently inhibits lung metastasis. *Molecular Therapy, 20*(3), 609–615.

Yu, B., Tai, H. C., Xue, W., Lee, L. J., & Lee, R. J. (2010). Receptor-targeted nanocarriers for therapeutic delivery to cancer. *Molecular Membrane Biology, 27*(7), 286–298.

Zhang, W., Shi, Y., Chen, Y., Hao, J., Sha, X., & Fang, X. (2011). The potential of Pluronic polymeric micelles encapsulated with paclitaxel for the treatment of melanoma using subcutaneous and pulmonary metastatic mice models. *Biomaterials, 32*(25), 5934–5944.

Zheng, L., Gou, M., Zhou, S., Yi, T., Zhong, Q., Li, Z., et al. (2011). Antitumor activity of monomethoxy poly(ethylene glycol)–poly (epsilon-caprolactone) micelle-encapsulated doxorubicin against mouse melanoma. *Oncology Reports, 25*(6), 1557–1564.

Zhou, T., Xiao, C., Fan, J., Chen, S., Shen, J., Wu, W., et al. (2013). A nanogel of onsite tunable pH-response for efficient anticancer drug delivery. *Acta Biomaterialia, 9*(1), 4546–4557.

Zhu, S., Lansakara-P, D. S., Li, X., & Cui, Z. (2012). Lysosomal delivery of a lipophilic gemcitabine prodrug using novel acid-sensitive micelles improved its antitumor activity. *Bioconjugate Chemistry, 23*(5), 966–980.

Bioinformatics in Skin Cancer: A System Biology Approach to Understanding the Molecular Mechanisms and It's Regulations

Manish Kumar Tripathi, Jitendera Sinha, Sushant Kumar Srivastava, and Dhruv Kumar

Abstract

In last few years, average life expectancy of humans has increased globally. Exposure to hazardous chemicals, ultraviolet (UV) radiation and other carcinogens has also increased the cancer burden in humans. Skin cancer is mainly associated with the DNA damage because of the prolonged exposure to UV radiation. Several structural changes, mutations and amplifications of oncogenes have been reported in skin cancer. The continuous development of bioinformatics approaches, next-generation sequencing, proteomics, transcripts, and epigenetic analysis has increased the understanding of cancer progression and molecular mechanisms and to design appropriate drug for the diseases. In this chapter, we have discussed the importance of bioinformatics in understanding the molecular mechanisms of skin cancer progression, related signaling pathways, data analysis, and drug development.

Keywords

Bioinformatics · Cancer · Cancer informatics · Melanoma · Squamous cell carcinoma

M. K. Tripathi · S. K. Srivastava
Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi, India

J. Sinha
Amity Institute of Neuropsychology and Neurosciences (AINN), Amity University, Noida, India

D. Kumar (✉)
Amity Institute of Molecular Medicine & Stem Cell Research Lab, Amity University, Noida, India
e-mail: dkumar13@amity.edu

A. Dwivedi et al. (eds.), *Skin Aging & Cancer*,
https://doi.org/10.1007/978-981-13-2541-0_10

1 Introduction

Genomic instability in normal cells initiates tumor progression. Tumors accumulate huge numbers of chromosomal abnormalities, mutations, and structural changes. All of these aberrations are thought to be critical for tumor growth and progression. The most forthright method for determining the progression of these aberrations is to evaluate multiple samples from the same individual, such as primary and metastatic samples (Frumkin et al. 2008; Gerlinger et al. 2012), sub-clonal populations (Campbell et al. 2008), or different portions of the same tumor (Navin and Hicks 2010; Siegmund et al. 2009).

Data collection during tumor progression is very important. It is very difficult to collect data on multiple time points of an individual tumor during tumor progression whereas data collection at single time point from multiple individuals is easy. There has been a great deal of interest in identifying driver genes, mutations, and events based on the frequency of their occurrence across cancer patients (e.g., Beroukhim et al. 2007; Cancer Genome Atlas Research Network 2008, 2011; Taylor et al. 2008). Numerous bioinformatics and statistical methodologies thoroughly analyze frequencies of abnormalities to determine the mutations, single nucleotide variation, copy number abnormalities, and other types of genomic alterations in cancer samples. Several developments have been made in the field of bioinformatics to understand the cancer progression and identification of structural abnormalities in chromosomes and to predict drug molecules for the driver targets.

This chapter is an overview of skin cancer and their characteristics and currently used bioinformatics approaches to understand the molecular mechanism and therapeutics in skin cancer. We conclude with several prospects of the future in skin cancer therapeutics using bioinformatics approach.

2 Skin Cancer

Skin cancer arises from the genomic instability in normal skin cells which leads to the abnormal growth of skin cells. It happens when DNA damage occurs to skin cells which is mainly caused by ultraviolet radiation (UV radiation) from sunshine. The change in DNS strands and DNA damage leads to the mutations/genetic defects which further lead the skin cells to multiply rapidly in uncontrolled manner and form malignant tumors. The most common types of skin cancers are squamous cell carcinoma, basal cell carcinoma, and melanoma (Orthaber et al. 2017). Squamous cell carcinoma is the most common skin cancer than melanoma and other types whereas melanoma is less common but has much more to invasion properties to other parts of the body. Melanoma cancer is developed in skin melanocytes which are sporadically exposed to the sun radiation. Among all skin cancers, melanoma causes most deaths (D'Orazio et al. 2013; Simões et al. 2015).

3 Cancer Informatics

In last ten decades scientist and clinicians have tried to understand the molecular mechanisms of cancer progression and to develop potential therapeutic approach for cancers. Significant national and international investments in cancer research have dramatically increased our understanding of the disease progression, leading to improvements in cancer diagnosis, treatment, and management, resulting in improved outcomes for many patients. Bioinformatics and cancer informatics have played a crucial role in understanding the molecular mechanisms of disease progression, specific mutation, structural changes, gene expression, identification of driver genes, and disease progression (Gligorijevic and Przulj 2015; Bumgarner 2013).

In melanoma, with the help of bioinformatics, several potential drugable targets and specific mutation have been identified including V600E mutation in the BRAF gene, expression of cyclins, kinases, and other oncogenes. BRAF is a serine/threonine protein kinase activating the MAP kinase/ERK signaling pathway, and both BRAF and MEK inhibitors, such as dabrafenib and vemurafenib, have shown significant responses in patients carrying the mutation. The accelerating progress in the development of diagnostics and therapeutic technologies (next-generation sequencing technology, cancer proteomics, cancer metabolomics, cancer transcriptomics, cancer epigenomics, and development in imaging technologies) has made very easy to understand the molecular mechanisms of cancer research (Hoek et al. 2004; Haqq et al. 2005; Gallagher et al. 2005; Wachsman et al. 2011).

4 Bioinformatics in Skin Cancer

4.1 Next-Generation Sequencing

Next-generation sequencing is a high-throughput technology of the modern era that conducts parallel sequencing of millions of DNA fragments. These kinds of technological advancement are gaining a lot of prominence in cancer investigations. The development of next-generation sequencing (NGS) has not only helped identify genetic variants but also represents an important aid in the study of epigenetics (DNAseq and ChIP-seq of histone methylation marks), transcriptional regulation, and splicing (RNAseq). The combined power of such genomic data provides a more complete definition of cancer genomes.

4.2 Microarray

Bioinformatics approach gains a major incentive in recent decades with the development of omics techniques. The DNA microarray is the most widely used omics technique used in the cancer diagnosis, to reveal the vast number of gene expression

alterations associated with the human malignancies. In microarray technology, the DNA probes are immobilized on a surface (called a chip or slide) which is made of glass or nylon. The samples like total bulk DNA or RNA from the specimen are labeled on this chip and the hybridization principles are used to measure the individual genes in a sample. DNA microarray methodology in skin cancer such as Affymetrix and Illumina is highly used because they simultaneously measure the expression of the entire genome.

Directly accessible skin was the first organ studied using the omics approach. "Skinomics" is a branch in which we study the dermatology and skin biology of the human beings. Studies are also focused to a significant extent to understand the skin cancer or melanoma. Widely skinomics studies involving 20 different countries have enabled us to identify the susceptible loci in the human genome and identified the genes which have a significant role in the pathology of this disease. Skin cancers, melanomas, and basal and squamous cell carcinomas are intensely investigated by DNA microarray technology because they are very recurrent and can be fatal. Epidermal stem cell-specific genes are identified using the DNA microarray technology and also the characterization of transcriptional changes which happen during the process of epidermal differentiation. The effects of epidermal and barrier disruption have also been characterized in some studies. The effect of ultraviolet (UV) light, hormones, immunomodulatory and inflammatory cytokines, chemokines and growth factors, etc., was also analyzed in cultured epidermal keratinocytes in many studies. Microarray analysis in nonmelanoma skin cancer analyzes the gene expression alteration and identified the biological significance of these alterations.

Discoveries in the cell signaling mechanism help us to understand the mechanism that underlies melanoma progression. Targeting the MAPK pathway, especially Ras, Raf, MEK, and ERK pathway, has been targeted for cancer treatment. Many compounds are identified or designed for therapeutic effects using these targets and presently are in clinical trials or already approved for the therapy. Sorafenib, a multi-tyrosine kinase inhibitor, was the first B-Raf inhibitor reached in a clinical trial and approved for the renal cancer treatment, but this compound failed to show antitumor activity in advanced melanoma cases. Some inhibitors also develop targeting specific BRAFv60E, namely, dabrafenib (GSK2118436) and vemurafenib (PLX4032); of this vemurafenib was the first US Food and Drug Administration (FDA)-approved B-Raf inhibitor for melanoma treatment. Some other pathways are also discovered and targeted, namely, Wnt, NF-κB, JAK-STAT pathway, transforming growth factor-β (TGF-β) pathway, and NOTCH pathway for the treatment of melanoma cancer. In Wnt signaling pathway mainly Frizzled receptors and DVL are considered as a target for the drug discovery against cancer treatment. Using bioinformatics approaches some natural compounds, namely, chalcones, derricin, and derricidin, are identified as Wnt signaling pathway inhibitors. Effects of these drugs are characterized by the reduced amount of nuclear β-catenin in effected cells.

4.3 Proteomics

Proteomics is defined as the large-scale study of proteome and their function. Progressively increasing the high-throughput generations of large proteome data has led to the bioinformatics to be an emerging and powerful tool for the study of these data. Bioinformatics approaches present novel algorithms to accomplish and analysis of huge generated heterogeneous proteomics data (Vihinen 2001). To search the protein ontology, a combination of databases is used to gain information for protein annotation, subcellular location, protein-encoding genes, and molecular function of the protein. Proteome (http://www.proteom.com/databases/HumanPD/reports) database represents the information of human proteome. In addition, protein-protein interaction and their network can be drawn based on the list of genes using the STRING database (http://string.embl.de/). Peptide and its amino acid sequence are also determined with the help of computational algorithms, namely, MASCOT, Protein Prospector, SEQUEST, and Paragon. Thus, proteome-based bioinformatics methodologies enable us to identify and validate the relevant cancer biomarkers. The altered expression state of these biomarkers assists us to correlate the biological behavior or clinical outcome of a particular trait. In the human being the skin cancer biomarkers used for the diagnostic study are, namely, Human Melanoma Black-45 (HMB-45), microphthalmia transcription factor, tyrosinase, Melan-A, and S100 as well as several newer ones (Weinstein et al. 2014). First proteomics study in skin cancer was done in 2000 to compare the proteome of human epidermal stem cells and their differentiated daughters (transit-amplifying cells). A computational study focusing on melanoma subtypes identified proteins, namely, vimentin, nestin, fibronectin, annexin A1, dipeptidyl peptidase IV, and histone H2A1B, as potential biomarkers for distinguishing melanoma subtypes (Qendro et al. 2014).

4.4 Genomics

The genome is defined as the complete set of sequence in the genetic material of an organism. Advancement in genomics and growth of genome data in the past decades enable us to understand the biological unpinning of information in skin cancer. During different developmental stages in cancer, the diseased cells show a series of changes that lead to the alteration of their metabolism (Mount and Pandey 2005). The bioinformatics tools and techniques help us to analyze and gain the information of the sequence and molecular data changes that will happen during this sequential progression. It also enables us to collect information on all the human gene and proteins. Various online resources are also available that help us to collect the information in cancer studies like The Cancer Genome Atlas (https://cancergenome.nih.gov/), International Cancer Genome Consortium (http://icgc.org/), etc., which focus

on identifying the links between cancer and genomic variation. According to TCGA database the genes, namely, TTN, MUC16, BRAF, DNAH5, PCLO, LRP1B, CSMD1, ADGRV1, DNAH7, ANK3, and APOB, were found to be highly mutated in skin cancer patients. Using computational approaches genes, namely, CXCL8, STAT1, CCL27, and IGF1R, were identified which play a role in the pathogenesis of melanoma (Zhang et al. 2017). It has been also found that the RAS pathway shows a prominent role in the pathogenesis of several skin cancer types.

4.5 Transcriptomics

A study of transcriptome which includes the complete set of mRNA and/or noncoding ribonucleotide (ncRNA) transcript produced by a particular cell, cell type, or organism is called transcriptomics. It helps in the screening of mutants and functional analysis of genes and also in the understanding of molecular mechanism of skin cancer. Bioinformatics tools help in the analysis and quantification of the transcriptome data for RNA modifications, RNA-protein interactions, noncoding RNA, and RNA structure (Han et al. 2015). For differential expression analysis of genes, transcriptome data tools, namely, edgeR, DESeq, NOIseq, SAMseq, Cuffdiff, and EBSeq, are widely used (Yang and Kim 2015). Transcriptome data help in the identification of the novel gene, expression, and splicing analysis. In skin, photocarcinogenesis is induced by the exposure of ultraviolet radiation (UVR) which affects the transcriptional instability (Shen et al. 2016). Transcriptome data analysis using bioinformatics method helps in the identification of mutated and tumor-specific disruptive genes, namely, ANKRA2, GTF2H5, STOML1, NUP37, PPP1R26, and TAF1L, in cell adhesion pathway (Zhang et al. 2013). Some gene expression profiling studies using insilico methods also identify the potential blood markers, namely, neudesin neurotrophic factor, ADAM-like decysin 1, apolipoprotein L6, MMP19, C-X-C motif chemokine ligand (CXCL)8, basic immunoglobulin-like variable motif containing, and CXCL16, which were used in the detection of early transformation of melanoma cases (Ortega-Bernal et al. 2018).

4.6 Epigenomics

Epigenetics refers to heritable changes in gene expression that are not caused by changes in the genomic DNA sequence. The principal epigenetic modification includes the changes in DNA and histone. The epigenetic regulation associated with these modifications causes the transcriptional activation or inactivation of genes. At cellular level, major epigenetic changes observed include the DNA methylation, histone modifications, and miRNA gene regulation (Saha et al. 2013). In epigenetic modification methylation at lysine 4, 36, and 79 residues on histone 3 (H3K4,

H3K36, and H3K79) and trimethylation at lysine 9 and 27 residues on histone 3 (H3K9me3 and H3K27me3) were observed. These epigenetic changes have been found to be associated with the cutaneous melanoma tumorigenesis and progression (Greenberg et al. 2014).

Various approaches of computational bioinformatics are available which help in the annotation of epigenome network in biomedical research. Various epigenetic databases are also available such as Catalogue of Somatic Mutations in Cancer (COSMIC), MethDB, MethBank, REBASE, Histone Database, HIstome, etc., which help the researcher to gain information about the epigenetic modification pattern like methylation and histone modifications (Ruskin and Barat 2018). Bioinformatics methods, namely, artificial neural network (ANN), machine learning, and support vector machines (SVM), are widely used for the analysis of DNA methylation CpG island pattern analysis and histone modifications (acetylation, methylation, phosphorylation). Molecular modeling approaches, i.e., homology modeling, molecular docking, molecular dynamics simulation, and QSAR analysis, are widely used for the detection of histone modifications (Shokri-Gharelo and Ghorbani 2018). Modeling studies also help in the identification of various phytochemicals like flavonoids and polyphenols which have found to play a crucial role as chemopreventive and chemotherapeutics activity against the different pathological processes including the skin cancer. Phytocompounds, namely, curcumin and resveratrol, showed the anti-melanoma activity in melanoma cells via inhibiting the NF-κB pathway. Sulforaphane, another phytoconstituent, was also found to show anticancer activity aginst skin cancer by inhibiting the HDACs and DNA methylation (Penta et al. 2018). With the development of next-generation sequencing facility, it enhances the biomedical research and enables us to sequence multiple cancer-driving genes and detect mutants with improved sensitivity.

5 Computational Drug Designing for Skin Cancer

In the field of drug discovery with the advancement of novel techniques, molecular docking became an important method to identify the lead molecule against a particular target. Molecular docking is a method which is used to predict the preferred binding orientation of one molecule with respect to another when bound together in a stable complex and help to understand the action mechanism and knowledge about drug interaction mechanism. The docking methods can be used for validation with experimental data as well as to find new ligands for receptor molecules. The basic component of docking program is based on two steps commonly called as searching algorithm and energy scoring function. Generation of possible binding mode of the ligand in the binding site of receptor is done with the help of search algorithm and scoring function is used for quantifying the binding strength of ligand with the receptor.

Commonly used scoring functions are under three broad categories:

1. *Physical*: molecular mechanics force fields for the estimation of binding affinity.
2. *Empirical data*: functions fitted to experimental data which is based on the knowledge of known protein-ligand interaction.
3. *Knowledge-based methods*: capture the knowledge of receptor-ligand binding by statistical data alone. Protein-ligand interactions involve structural alterations in the receptor binding site and ligand. Thus, the model allows flexibility in both the ligands and the receptor. Docking program named AUTODOCK, GOLD, GLIDE, FLEX, etc., allows partial flexibility of the receptor and full flexibility of the ligand.

We performed molecular docking study using FDA-approved anticancer compounds (hydrochloride (Idarubicin), eribulin, eribulin mesylate, vinblastine, irinotecan, vincristine, tartrate (vinorelbine), trabectedin, vinorelbine, midostaurin) on melanoma target, namely, BRAF and MAPK3, to identify potential lead molecule for the therapeutic effect against skin cancer. The study showed that hydrochloride (Idarubicin) showed maximum binding affinity with MAPK3 and midostaurin with BRAF receptor and their docking energy was found to be −5.80 and −7.64 Kcal/mole. The docking energy and interacting residues of all other compounds are shown in the table. From the docked complex (Fig. 1), it revealed that hydrochloride (Idarubicin) shows important interaction with Leu50, Val58, Met118, Met121, Glu122, and Gly124 amino acid residues of MAPK3 receptor. Another inhibitor, namely, midostaurin, showed interaction with Ala481 and Cys534 amino acid residues of BRAF receptor (Table 1).

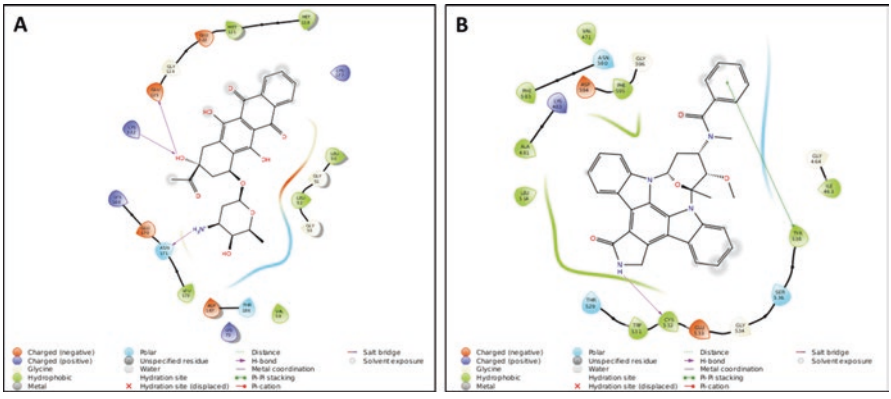

Fig. 1 Figure represents the molecular docking between selected FDA-approved compounds with MAPK3 BRAF. (**a**) Hydrochloride (Idarubicin) showed the maximum binding affinity (−5.80 Kcal/mol) with MAPK3 and (**b**) midostaurin showed the maximum binding affinity (−7.64 Kcal/mol) with BRAF receptor

Table 1 List of selected FDA-approved anticancer compounds docked with human MAPK3 and BRAF

S. no.	Name of protein (PDB ID)	Ligands	Binding energy (Kcal/Mole)	Interacting residues
1	MAPK3 (3FHR)	Idarubicin	−5.77	Leu50,Val58,Met118, Met121,Glu122,Gly124
		Hydrochloride (Idarubicin)	**−5.80**	**Leu50,Val58,Met118, Met121,Glu122,Gly124**
		Eribulin	−4.70	Leu50,Gly124
		Eribulin mesylate	−4.70	Leu50,Gly124
		Vinblastine	−4.51	Leu50,Met121,Glu122,Gly124
		Irinotecan	−4.19	Leu50, Cys120,Met121,Glu122,Gly124
		Vincristine	−4.41	Leu50, Cys120,Met121,Glu122,Gly124
		Tartrate (vinorelbine)	−4.17	Leu50, Cys120,Met121,Glu122,Gly124
		Trabectedin	−3.76	Leu50, Cys120,Met121,Glu122,Gly124
		Vinorelbine	−3.31	Leu50, Cys120,Met121,Glu122,Gly124
		Midostaurin	−1.75	Leu50, Cys120,Met121,Glu122
2	BRAF (4R5Y)	Idarubicin	−7.11	Glu501,Leu505,Leu514
		Hydrochloride (Idarubicin)	−7.118	Glu501,Leu505,Leu514
		Eribulin	–	–
		Eribulin mesylate	–	–
		Vinblastine	−2.49	Glu501,Leu505,
		Irinotecan	−5.46	Ala481,Glu501,Leu505,Leu514,Cys534
		Vincristine	−2.80	Glu501
		Tartrate (vinorelbine)	−2.05	Glu501,
		Trabectedin	–	–
		Vinorelbine	−3.56	Cys534
		Midostaurin	**−7.64**	**Ala481,Cys534**

6 Conclusion

Nowadays cancer data collection, storage, and proper data analysis are of major concern. Bioinformatics plays an important role in the proper cancer data management and cancer data analysis. With the help of bioinformatics tools, scientists are able to properly analyze the big data set and to analyze gene expression and structural changes and to identify specific mutations in skin cancer, which further help in designing specific inhibitors for certain driver genes and against specific mutations associated with cancer progression.

References

Beroukhim, R., et al. (2007). Assessing the significance of chromosomal aberrations in cancer: Methodology and application to glioma. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 20007–20012.

Bumgarner, R. (2013). Overview of DNA microarrays: Types, applications, and their future. *Current Protocols in Molecular Biology*. Chapter(22): Unit 22.1. https://doi.org/10.1002/0471142727.mb2201s101.

Campbell, P. J., et al. (2008). Subclonal phylogenetic structures in cancer revealed by ultra-deep sequencing. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 13081–13086.

Cancer Genome Atlas Research Network. (2008). Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature, 455*(7216), 1061–1068.

Cancer Genome Atlas Research Network. (2011). Integrated genomic analyses of ovarian carcinoma. *Nature, 474*, 609–615.

D'Orazio, J., Jarrett, S., Amaro-Ortiz, A., & Scott, T. (2013). UV radiation and the skin. *International Journal of Molecular Sciences, 14*(6), 12222–12248.

Frumkin, D., et al. (2008). Cell lineage analysis of a mouse tumor. *Cancer Research, 68*, 5924–5931.

Gallagher, W. M., Bergin, O. E., Rafferty, M., Kelly, Z. D., Nolan, I. M., Fox, E. J., et al. (2005). Multiple markers for melanoma progression regulated by DNA methylation: Insights from transcriptomic studies. *Carcinogenesis, 26*, 1856–1867. Epub 2005 Jun 15.

Gerlinger, M., et al. (2012). Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *The New England Journal of Medicine, 366*, 883–892.

Gligorijevic, V., & Przulj, N. (2015). Methods for biological data integration: Perspectives and challenges. *Journal of the Royal Society Interface, 112,* 1–19. pii:20150571. https://doi.org/10.1098/rsif.2015.0571.

Greenberg, E. S., Chong, K. K., Huynh, K. T., Tanaka, R., & Hoon, D. S. (2014, January 28). Epigenetic biomarkers in skin cancer. *Cancer Letters, 342*(2), 170–177.

Han, Y., Gao, S., Muegge, K., Zhang, W., & Zhou, B. (2015). Advanced applications of RNA sequencing and challenges. *Bioinformatics and Biology Insights, 9*(Suppl 1), 29–46.

Haqq, C., Nosrati, M., Sudilovsky, D., Crothers, J., Khodabakhsh, D., Pulliam, B. L., et al. (2005). The gene expression signatures of melanoma progression. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 6092–6097. Epub 2005 Apr 15.

Hoek, K., Rimm, D. L., Williams, K. R., Zhao, H., Ariyan, S., Lin, A., et al. (2004). Expression profiling reveals novel pathways in the transformation of melanocytes to melanomas. *Cancer Research, 64*, 5270–5282.

Mount, D. W., & Pandey, R. (2005). Using bioinformatics and genome analysis for new therapeutic interventions. *Molecular Cancer Therapeutics, 4*(10), 1636–1643.

Navin, N. E., & Hicks, J. (2010). Tracing the tumor lineage. *Molecular Oncology, 4*, 267–283.

Ortega-Bernal, D., La Rosa, C. H. G., Arechaga-Ocampo, E., Alvarez-Avitia, M. A., Moreno, N. S., & Rangel-Escareño, C. (2018, August). A meta-analysis of transcriptome datasets characterizes malignant transformation from melanocytes and nevi to melanoma. *Oncology Letters, 16*(2), 1899–1911.

Orthaber, K., Pristovnik, M., Skok, K., Perić, B., & Maver, U. (2017). Skin Cancer and its treatment: Novel treatment approaches with emphasis on nanotechnology. *Journal of Nanomaterials*, 1–21.

Penta, D., Somashekar, B. S., & Meeran, S. M. (2018, January). Epigenetics of skin cancer: Interventions by selected bioactive phytochemicals. *Photodermatology, Photoimmunology & Photomedicine, 34*(1), 42–49.

Qendro, V., Lundgren, D. H., Rezaul, K., Mahony, F., Ferrell, N., Bi, A., Latifi, A., Chowdhury, D., Gygi, S., Haas, W., Wilson, L., Murphy, M., & Han, D. K. (2014, November 7). Large-scale proteomic characterization of melanoma expressed proteins reveals nestin and vimentin as bio-

markers that can potentially distinguish melanoma subtypes. *Journal of Proteome Research, 13*(11), 5031–5040.

Ruskin, H. J., & Barat, A. (2018). Recent advance in computational epigenetics. *Advances in Genomics and Genetics, 8*, 1–12.

Saha, K., Hornyak, T. J., & Eckert, R. L. (2013, October). Epigenetic cancer prevention mechanisms in skin cancer. *The AAPS Journal, 15*(4), 1064–1071.

Shen, Y., Kim, A. L., Du, R., & Liu, L. (2016, September 19). Transcriptome analysis identifies the dysregulation of ultraviolet target genes in human skin cancers. *PLoS One, 11*(9), e0163054.

Shokri-Gharelo, R., & Ghorbani, R. (2018). Computational-based approaches in epigenetic research: Insights from computational tools, mathematical models, and machine learning methods. *Journal of Genetics and DNA Research, 2*(1), 106.

Siegmund, K. D., et al. (2009). Inferring clonal expansion and cancer stem cell dynamics from DNA methylation patterns in colorectal cancers. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 4828–4833.

Simões, M. C. F., Sousa, J. J. S., & Pais, A. A. C. C. (2015). Skin cancer and new treatment perspectives: A review. *Cancer Letters, 357*(1), 8–42.

Taylor, B. S., et al. (2008). Functional copy-number alterations in cancer. *PLoS One, 3*, e3179.

Vihinen, M. (2001). Bioinformatics in proteomics. *Biomolecular Engineering, 18*(5), 241–248.

Wachsman, W., Morhenn, V., Palmer, T., Walls, L., Hata, T., Zalla, J., et al. (2011). Noninvasive genomic detection of melanoma. *The British Journal of Dermatology, 164*, 797–806. https://doi.org/10.1111/j.365-2133.011.10239.x. Epub 2011 Mar 25.

Weinstein, D., Leininger, J., Hamby, C., & Safai, B. (2014, June). Diagnostic and prognostic biomarkers in melanoma. *The Journal of Clinical and Aesthetic Dermatology, 7*(6), 13–24.

Yang, I. S., & Kim, S. (2015, December). Analysis of whole transcriptome sequencing data: Workflow and software. *Genomics & Informatics, 13*(4), 119–125.

Zhang, Q., Zhang, J., Jin, H., & Sheng, S. (2013). Whole transcriptome sequencing identifies tumor-specific mutations in human oral squamous cell carcinoma. *BMC Medical Genomics, 6*, 28.

Zhang, Q., Wang, Y., Liang, J., Tian, Y., Yu Zhang, M. M., & Tao, K. (2017, July). Bioinformatics analysis to identify the critical genes, microRNAs and long noncoding RNAs in melanoma. *Medicine (Baltimore), 96*(29), e7497.

UV-R Interaction with Skin: Cases of Study

Gonzalo Gurrea Ysasi

Abstract

People developing their daily activities outdoors are likely to receive high doses of ultraviolet radiation (UV). In this chapter, three different cases of study on the effect of UV-R on skin have been analyzed: in the first one UV-R dose athletes from different sports (tennis, hiking, and running) in their workout have been studied. The second case is focused on the maximum values UV-R dose can reach for construction workers in Valencia (Spain) (39°280 N, 0°220 W). Finally, the last case of study focuses on UVER (erythemal ultraviolet radiation) received on a driver and passenger position inside a vehicle. In the three cases VioSpor blue line dosimeters (with a response profile close to that of human skin) have been employed for measurements.

Keywords

Erythema · UV-R dose · UVER

1 Personal UV Exposure for Different Outdoor Sports

1.1 Introduction

Nowadays the practice of sport and, more specifically, outdoor sport, is becoming very popular among people. It is true that doctors and other health organizations vouch this kind of activities because of its beneficial effects not only on cardiovascular system but also on psychological aspects. However, it is also known that

G. G. Ysasi (✉)
Departamento de Construcciones Arquitectónicas, Universitat Politècnica de València, Valencia, Spain
e-mail: gongurys@csa.upv.es

A. Dwivedi et al. (eds.), *Skin Aging & Cancer*,
https://doi.org/10.1007/978-981-13-2541-0_11

people practicing outdoor sports may be affected by high doses of solar UV-R radiation depending on the season and the time of the day of their training sessions. For example, in summer days the influence of solar UV-R may be harmful because of the appearance of melanoma on skin of marathon runners, cyclists, mountain guides, or people who play golf (Ambros-Rudolph et al. 2006; Richtig et al. 2008; Williams et al. 1989; Lichte et al. 2010).

1.2 Personal UV-R Dosimeters

In this study, for measuring the cumulative solar erythemal UV exposure (UVER), VioSpor blue line dosimeter type I (VioSpor, BioSense. Bornheim, Germany) (Biosense Laboratories n.d.; Furusawa et al. 1998) was used. The estimations depend on the generation of spore films (with DNA of *Bacillus subtilis*) secured by a channel framework with optical reaction equal to human skin agreeing the International Commission on Illumination (CIE) reference range (C.I.E. Commission Internationale de l'Eclairage 1998). The units used are expressed in standard erythema dose (SED), being 1 SED the effective erythemal exposure of 100 J m^{-2} when weighted with the CIE erythemal spectrum. The measurement error is ±10%, according to the manufacturer (Biosense Laboratories n.d.).

1.3 UV Exposure Limits Considered

An individual day by day UV-R limit of 30 J m^{-2} dose is the proposed from the International Commission on Non-Ionizing Radiation Protection (ICNIRP) (International Commission on Non-Ionizing Radiation Protection (ICNIRP) 2010). This is known as the exposure limit (EL) in 8 h for unprotected skin using the American Conference of Government Industrial Hygienists (ACGIH) action range. In this text the link between the two spectra is effective exposure CIE = 3.63 × effective exposure ACGIH (Moehrle et al. 2003). The result is, by using the CIE spectrum, a value of 109 J m^{-2} (1.1 SED) per 8-h period. This EL can be increased until a value of 5 SED for skin phototype III without adaptation to the sun and even a value of 12 SED for sun-adapted skin phototype III.

1.4 Groups of Study

In this chapter UV-R exposure received by tennis players, hikers, and runners during their training schedules is studied (Serrano et al. 2014).

1.4.1 Hikers

It is important to distinguish, first, the term "walking" versus "hiking." While the first one refers to shorter and urban walks with a length of maximum 1 h, the second one refers to a longer walk, typically in the countryside, with a length that can vary

from 4 to more than 7 h in a day. That is the reason why this activity is likely to be more harmful when talking about UV-R exposure for human skin.

In order to analyze UV-R received by this group, four people (two instructors and two students) were selected to take part of the study.

The study was carried out during six hikes during the hottest time of the year (June (12th), July (3rd, 8th, and 9th), and August (11th and 12th) in 2011). The first two walking journeys were near Valencia (Spain), the third one (which lasted 2 days) was in Cauterets (France), and the last hike (lasting 2 days) was on La Palma (Spain).

Ambient UV Exposure

For the journeys in Valencia, the erythemal UV-R was acquired from the Valencian territorial government's (GV) UVB measurements (Programa meteorología de la Fundación Centro de Estudios Ambientales del Mediterráneo n.d.) which is composed of two radiometers YES UVB-1 with a range from 280 to 400 nm. For the rest of the hikes, measurements were obtained by means of FastRT 2.3 program (Engelsen and Kylling 2005).

Results

As shown in Table 1, people involved in hiking activities got an average UV-R value of 8.1 SED [1.8–19.5 SED]. Also, the time to erythema (t_e in minutes) to surpass the EL for the time of study was in the range of 16 and 76 min. It is appeared in Table 2. In this chapter exposure ratio has been defined as the proportion between the UV measurement on a particular place on the body and the ambient UV dose received on horizontal position.

Table 1 UV exposure and ER for hikers

	UV exposure				
	Median (SED)	Maximum (SED)	Minimum (SED)	Mean time spent outdoors (h)	Exposure ratio % median (Max–Min)
12/06/2011	7.9	9.4	7.9	4.5	16.9 (20.1–16.7)
03/07/2011	3.9	4.6	1.8	4.3	18.8 (22.5–8.9)
08/07/2011	14.3	19.5	8.6	6.8	24.6 (33.5–14.7)
09/07/2011	6.4	10.2	6.2	9.5	10.2 (16.1–9.8)
11/08/2011	9.2	12.8	6.1	6.5	14.0 (19.6–9.3)
12/08/2011	9.7	12.1	6.0	6.5	16.2 (20.2–10.0)
Mean for the hiking group	8.1	19.5	1.8	6.4	16.4 (33.5–8.9)

Table 2 Time needed to exceed the exposure limit recommended by ICNIRP for hikers

12/06/2011 $t_{e\text{-}UVR}$ (minutes)	3/07/2011 $t_{e\text{-}UVR}$ (minutes)	8/07/2011 $t_{e\text{-}UVR}$ (minutes)	9/07/2011 $t_{e\text{-}UVR}$ (minutes)	11/08/2011 $t_{e\text{-}UVR}$ (minutes)	12/08/2011 $t_{e\text{-}UVR}$ (minutes)
58	76	22	39	64	16

1.4.2 Tennis Players

Similar study was developed with another kind of sport, in this case tennis. The place was Club de Tenis Valencia (0°22′W, 39°28′N, sea level) and took place during summer season (12th and 27th June and 5th, 14th, and 20th July 2011). In order to be as realistic as possible, two different tennis courts, each one with a different orientation, were selected. Median UV-R exposure was 7.5 SED [2.0–13.8 SED] and they exceeded the EL dose in 20 min.

Ambient Exposure

Ambient erythemal UV-R measurements were acquired from Valencia station GV UVB measurement network (Programa meteorología de la Fundación Centro de Estudios Ambientales del Mediterráneo n.d.).

Results

In the case of tennis players, an average UV-R value of 7.5 SED [13.8–2.0] was obtained, as can be seen in Table 3. The average value for ER was of 15.0 [3.8–26.6]. With respect to the time until UV-R exposure limit is exceeded, it has been calculated that, as shown in Table 4, a tennis player may exceed the limit recommended value in about 20 min.

1.4.3 Runners

Apart from tennis and hiking groups, another fashionable and likely to receive high amounts of UV dose are runners. In order to analyze UV dose received by them, in his study Universitat Politècnica de València (UPV) running club has taken part from March to November 2011 during the running season and involving several races at different geographical locations. Due to the fact that the study covers a wide range of months, the value of solar zenith angle also had important differences, from a maximum of 55.4 degrees in November to a minimum of 16.4 degrees in July. The ambient UV-R measurements were acquired from Valencia station

Table 3 UV exposure and ER for tennis players

	UV exposure				
	Median (SED)	Maximum (SED)	Minimum (SED)	Mean time spent outdoors (h)	Exposure ratio % median (Max–Min)
12/06/2011	7.4	9.8	3.6	4	14.9 (19.6–7.2)
27/06/2011	7.5	8.5	5.0	4	13.7 (15.5–9.1)
05/07/2011	8.0	9.6	4.8	4	16.3 (19.5–9.8)
14/07/2011	8.3	10.6	6.5	4	18.2 (23.4–14.4)
20/07/2011	9.0	13.8	2.0	4	17.4 (26.6–3.8)
Mean for the tennis player group	7.5	13.8	2.0	4	15.0 (26.6–3.8)
Coaches	7.5	13.8	2.0	4	15.0 (26.6–3.8)
Learners	7.9	10.7	4.8	4	15.0 (20.7–9.8)

Table 4 Time needed to exceed the exposure limit recommended by ICNIRP for tennis players

12/06/2011 $t_{e\text{-}UVR}$ (minutes)	27/06/2011 $t_{e\text{-}UVR}$ (minutes)	5/07/2011 $t_{e\text{-}UVR}$ (minutes)	14/07/2011 $t_{e\text{-}UVR}$ (minutes)	20/07/2011 $t_{e\text{-}UVR}$ (minutes)
20	20	22	23	22

Table 5 UV exposure and ER for runners

Subject/number of running races completed	Time spent outdoors (h)	UV exposure (SED)	SED/hours outdoors	Exposure ratio % median (Max–Min)	SED/ competition day
1/08	16.0	17.1	1.1	6.9	2.1
2/11	21.3	11.6	0.6	3.1	1.1
3/11	22.8	23.8	1.0	7.0	2.2
4/07	16.3	14.6	0.9	5.8	2.1
5/10	22.8	9.3	0.4	2.9	0.9
6/12	18.3	16.3	0.9	4.3	1.4
7/04	8.8	11.3	1.3	8.8	2.8
Median for all runners	18.3	14.6	0.9	5.8 (14.1–0.7)	2.1

GV UVB measurement network, as in the case of hikers and tennis players (Programa meteorología de la Fundación Centro de Estudios Ambientales del Mediterráneo n.d.).

Results

As shown in Table 5, the median UV-R exposure during the circuit was 14.6 SED.

1.5 Discussion

If EL recommended by ICNRP for non-sun-adapted skin type III (5 SED) is considered, it is shown that the UV-R dose resulting from this study surpasses this limit in the case of hikers and tennis players (averaged value of 8.1 SED and 7.5 SED in each case). Therefore, for these sports groups highly recommended is the use of protective measures in order to avoid high-dose values for people involved in these activities.

Despite the fact that it should be imprecise to make a comparison between results from these two groups (tennis players and people involved in hiking), it is shown that the first ones receive 2.0 SED while the second ones only a value of 1.3 SED. The reason may be the fact that tennis court that was object of this study was placed in a non-protected area. In other words, no physical barriers surrounded it in order to avoid the sun. In the case of hikers, most of the walks take place in the mountains or in the countryside, normally full of trees.

Regarding runners, due to the fact that is a nonhomogeneous group, because participants didn't run in the 12 races that formed the complete circuit, it should be incorrect comparing UV-R dose received for each participant. However, dose per hour is likely to be compared.

Taking a look at the UV-R averaged value for this group, 2.1 SED, is easy to see that doesn't pass the EL of 5 SED. The reason may be the fact that the race circuit was complete during the whole year (including summer but mainly winter and spring) and also the beginning of most of the races was very early in the morning when the sun has a very large value of zenith angle.

According to the results of this study, sportsmen and women involved in outdoor sports, like tennis, running, or hiking, may spend several hours per day during their workout or in a competition. In some cases, they may be more than 9 h exposed to solar UV-R. This can result in a high risk to produce erythema on their skin which in some cases is able to derive into skin cancer. As a result, it is highly recommended, and it should be advised by public organizations to use several measures in order to avoid the harmful effect of the sun, mainly in the midday hours and in hottest months of the year. The use of caps, sunglasses, and sun cream (factor higher than 30) should be highly recommended.

2 Maximum Personal UV Exposure for Construction Workers

2.1 Introduction

Another group potentially affected by high UV-R exposures is construction workers. When working in a construction, it is usual for them to have to spend several hours per day outdoors (with the corresponding risk to high doses of UV-R). The parts of the body main affected are usually the face and neck.

In this study, a static mannequin has been used in order to register the highest values of UVER dose that a laborer could receive while working. As a result, we can analyze the worst effects that UV-R can have on this particular group if they are not correctly protected (Blanca et al. 2015).

Skin phototype considered for construction workers in Valencia has been II and III.

2.2 Personal UV-R Dosimeters

The kind of dosimeters used in this study was the same as in the rest of groups (tennis players, runners, or hikers) (Biosense Laboratories n.d.; Furusawa et al. 1998). Dosimeters were placed in five positions of the mannequin, head, shoulders, forehead, and chest, as shown in Fig. 1.

Fig. 1 Mannequin used for measurements

2.3 Ambient UV-R Exposure

The ambient UV-R measurements were acquired from Valencia station GV UVB measurement network, as in the case of hikers, tennis players, and runners (Programa meteorología de la Fundación Centro de Estudios Ambientales del Mediterráneo n.d.).

2.4 UV-R Exposure Limits Considered

Following the ICNIRP report on exposure limit values (International Commission on Non-Ionizing Radiation Protection (ICNIRP) 2010), the average threshold exposure for non-adapted sun skin types II and III is 2 SED and 5 SED, respectively.

2.5 Subjects and Design

The study took place in Polytechnic University of Valencia (Spain) between 2012 December and 2013 July. The measurements were taken from 10:00 h to 16:00 h (local time). A static mannequin of 1.60 m height, facing the South, was used.

This orientation ensures the mannequin to receive the highest amount of radiation during the day in Valencia latitude.

The study was carried out in 2012 (27th, 28th December) and 2013 (8th, 10th, and 11th January; 21st March; 12th, 15th, 16th, and 17th April; 15th, 16th, 17th, 19th, and 22th July).

2.6 Results

In winter period, as it can be seen, the highest UV-R dose was found to be for the forehead (7.09 ± 2.30 SED). The reason may be the particular orientation of this position (almost perpendicular to sun position because solar height has a mean value of 18.7° in this time of the year).

During spring, the position of the sun changes in the sense of increasing its solar height until 44.6°. Therefore, horizontal dosimeters will be more perpendicular to sun position, reaching a value of 18.72 ± 5.32 SED on top of the head and 17.63 ± 4.57 SED and 16.70 ± 4.11 SED for right and on the left shoulder, respectively.

The results for summer show the same trend as explained above. The increase of solar height leads in the most UV-R dose for horizontal positions of mannequin (top of the head with a value of 31.20 ± 3.01 SED).

If we analyze the exposure ratio (ER), in order to compare the different results for the whole year, it can be said that, during winter, the highest value of ER is for forehead (100%), for spring period is for top of the head (75.54%), and in the summer period is for the top of the head (68.60%) (Tables 6, 7, and 8).

Table 7 UVER dose and ER for different mannequin positions (spring period)

Dosimeter site	Mean (SED)	Standard deviation (SED)	Mean SED per hour outdoors (range)	Mean time spent outdoors (h)	Exposure ratio (%)
Top of the head	18.72 (26.92–13.84)	5.32	3.54 (5.38–2.32)	5.4	75.54
Forehead	12.07 (14.79–10.25)	2.21	2.24 (2.96–1.95)	5.4	48.71
Right shoulder	17.63 (23.93–13.01)	4.57	3.32 (4.55–2.25)	5.4	71.14
Left shoulder	16.70 (23.4–12.42)	4.11	3.13 (4,46–2.25)	5.4	67.39
Chest	16.12 (20.56–12.89)	2.83	3.01 (4.11–2.45)	5.4	65.05

Table 6 UVER dose and ER for different mannequin positions (winter period)

Dosimeter site	Mean (SED) (range)	Standard deviation (SED)	Mean SED per hour outdoors (range)	Mean time spent outdoors (h)	Exposure ratio (%)
Top of head	6.39 (8.39–4.56)	1.54	1.03 (1.39–0.72)	6.23	90.16
Forehead	7.09 (10.92–4.76)	2.30	1.14 (1.82–0.76)	6.23	100.13
Right shoulder	6.22 (7.99–5.11)	1.11	1.00 (1.33–0.78)	6.23	87.84
Left shoulder	5.27 (7.02–3.76)	1.24	0.85 (1.12–0.61)	6.23	74.46
Chest	7.36 (9.06–6.46)	1.09	1.18 (1.44–0.99)	6.23	103.80

Table 8 UVER dose and ER for different mannequin positions (summer period)

Dosimeter site	Mean (SED)	Standard deviation (SED)	Mean SED per hour outdoors (range)	Mean lime spent outdoors (h)	Exposure ratio (%)
Top of the head	31.20 (34.54–27.85)	3.01	6.24 (6.91–557)	5	68.62
Forehead	9.81 (14.5–5.22)	4.37	1.96 (2.9–1.04)	5	21.57
Right shoulder	24.97 (29.09–21.32)	3.30	4.99 (5.82–426)	5	54.98
Left shoulder	23.93 (28.23–19.69)	3.34	4.79 (5.64–3.94)	5	52.64
Chest	16.73 (23.85–14.21)	4.06	3.35 (4.77–2.84)	5	36.81

2.7 Discussion

The fact of studying static positions for UV-R doses implies an increase in the mean values with respect to other studies involving dynamical positions.

Moreover, taking a look at the results obtained, it has been shown that for every season of the year and for every position analyzed, the UV-R dose received has always exceeded the limit recommended by ICNRIP for skin types I to IV.

During winter, the UV-R dose in chest position (7.36 SED) passes the limit recommend for skin type I/II by a factor of 3.68 and by a factor of 1.47 for skin type III/IV.

During spring time, it was measured an averaged UV-R dose for the head position of 18.72 SED which surpasses the recommended limit for skin type I/II by a factor of 9.36 and the limit for skin type III/IV by a factor of 3.74.

For summertime, it was measured an averaged UV-R dose for the head position of 31.20 SED surpassing the recommended limit for skin type I/II by a factor of 15.6 and the limit for skin type III/IV by a factor of 6.24.

Looking at the above values, it can be concluded that it should be necessary to include specific protective actions in order to avoid high UV-R doses.

3 Personal UV Exposure Received Inside a Car

3.1 Introduction

A particular and non-deep-studied case of study regarding UV-R doses on humans is the one related to people travelling inside vehicles. The reason is because the action of windows blocking the radiation is a given fact. However, it has been observed that this blocking action is deeper in shorter wavelengths but, when considering accumulated values during a long period of time, UV-R doses resulting need to be considered (Lavker et al. 1995; Lavker and Kaidbey 1997; Lowe et al. 1995; Sayre et al. 1997; Bisset et al. 1992). Additionally, there are many cases when people inside vehicles travel with opened windows and therefore the radiation is coming into the vehicle without any filter.

For example, people who work driving a bus or a taxi may spend more than 8 h inside the vehicle (Lowe et al. 1995). They may accumulate a considerable quantity of UV-R.

The difference between travelling with opened or closed windows has been considered in this study (Gurrea et al. 2018).

Also, and following the same methodology as in the above cases of study, it has been included the calculation of the time needed to produce an erythema on driver's skin.

3.2 Personal UV-R Dosimeters

For this study, the same kind of dosimeters as in the rest of the cases analyzed has been used (Biosense Laboratories n.d.; Furusawa et al. 1998). Figures 2 and 3 clearly show the different positions studied in both cases: driver and passenger. In each case four dosimeters have been used. The identification for the positions is the following: HD (horizontal driver), LD (lateral driver), VD (vertical driver), AD (armrest driver), HP (horizontal passenger), LP (lateral passenger), VP (vertical passenger), and AP (armrest passenger).

Two extra dosimeters have been placed outside the car, in horizontal and vertical position.

3.3 UV-R Exposure Limits Considered

Following the ICNIRP report on exposure limit values (International Commission on Non-Ionizing Radiation Protection (ICNIRP) 2010), the average threshold exposure for non-adapted sun skin types II and III is 2 SED and 5 SED, respectively.

3.4 Subjects and Design

The study took place in Paterna (Valencia) on cloudiness sky conditions from February to December 2009 (9:30 h to 15:00 h) (local time). The period of time when measurements were taken was February–March, May–June, July–September, and October–November–December, in 2009.

A three-door Peugeot 206 4–1360 cc., 1.4 L. 2000, was used for the study, measuring 3.82 m long, 1.67 m wide, and 1.44 m high.

For the development of the study a three-door car Peugeot 206 has been used.

In order to simplify the measurement conditions, but trying to emulate, as much as possible, the real situation, the car was placed with driver's window pointing to the sun. In order to do that, every 45 min, the car was turned about 10° following the sun.

As in previous cases, the time needed to produce an erythema on skin has been calculated knowing the exposure limit for the different types of skin, as it can be seen in Table 9 (Vanicek et al. 2000).

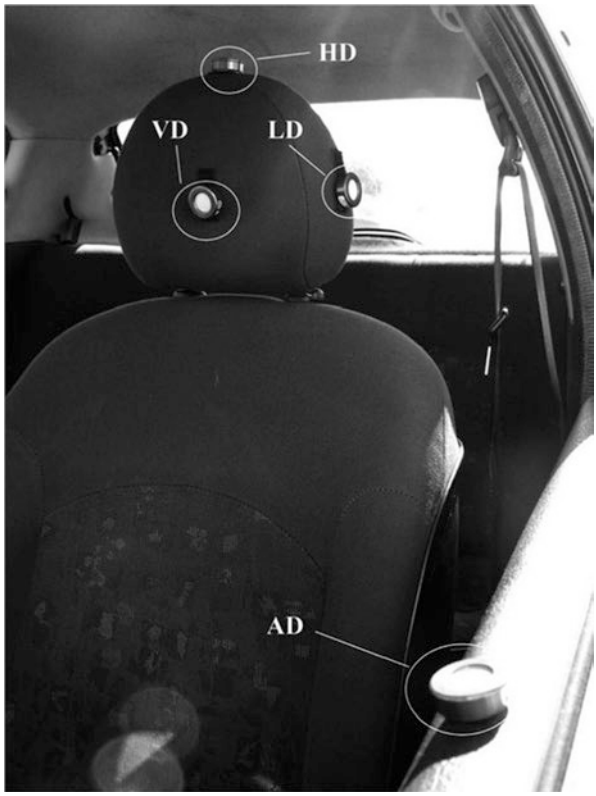

Fig. 2 Situation of dosimeters on driver place

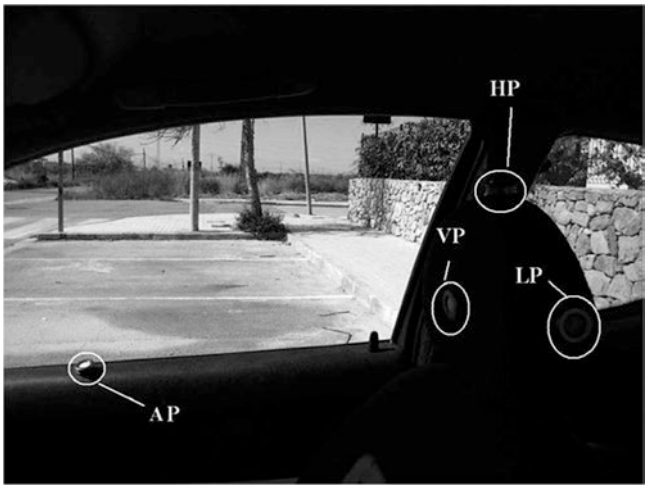

Fig. 3 Situation of dosimeters on passenger place

Table 9 UVER for each type of skin

Skin phototype	Dose UVER equivalent to one MED (J m^{-2})
I	200
II	250
III	350
IV	450
V	550
VI	650

Table 10 UVER during the February–March

Dosimeter position	UVER dose with closed windows (J m^{-2})	UVER dose with opened windows (J m^{-2})	UVER dose outdoors (J m^{-2})
HD	24.7	9.1	
LD	53.4	197.4	
VD	4.1	14	
AD	31	157.1	
HP	0.5	0.8	
LP	6.9	9.7	
VP	8.4	4.8	
AP	4.8	30.7	
H_{OUT}			161.8
V_{OUT}			171.2

Table 11 UVER during May–June

Dosimeter position	UVER dose with closed windows (J m^{-2})	UVER dose with opened windows (J m^{-2})	UVER dose outdoors (J m^{-2})
HD	0	16.8	
LD	69.3	183.8	
VD	0	14.5	
AD	87.9	324	
HP	14.5	6.1	
LP	0	17.4	
VP	11.6	20.6	
AP	26.4	298.4	
H_{OUT}			394.2
VOUT			227.7

3.5 Results

Taking a look at results from Tables 10, 11, 12, and 13, it is easy to conclude that, in the case of travelling with windows in closed position, the driver and also the passenger of a vehicle are effectively protected against UV-R. The value for UV-R dose is always lower than the limit recommended by ICNIRP. There is only one

Table 12 UVER during July–September

Dosimeter position	UVER dose with closed windows (J m^{-2})	UVER dose with opened windows (J m^{-2})	UVER dose outdoors (J m^{-2})
HD	7.5	90.2	
LD	103.6	333.5	
VD	71	144.3	
AD	135.5	393.6	
HP	76	76	
LP	92	109.1	
VP	55.6	48.1	
AP	75.1	254.9	
H_{OUT}			488.7
V_{OUT}			319

Table 13 UVER during October–November–December

Dosimeter position	UVER dose with closed windows (J m^{-2})	UVER dose with opened windows (J m^{-2})	UVER dose outdoors (J m^{-2})
HD	36.8	40.5	
LD	88.8	120.2	
VD	53.8	51.6	
AD	50	99	
HP	4.5	27	
LP	39.9	34.6	
VP	22.4	36.7	
AP	39.3	58.8	
H_{OUT}			133
V_{OUT}			135.2

exception: the case of the arm for driver position during summer period (July–September) reaching a value of 135.5 J m^{-2}.

However, in the case of travelling with completely opened windows, the situation changes dramatically. In both cases, driver and passenger position, the UV-R doses received may pass the limit recommended (by a factor of 3.1 in the case of lateral position and by a factor of 3.6 in the case of the arm, for summer period).

Taking a look at the results coming for Table 14, it should be stated that normally the time needed to appear an erythema on people travelling inside a car is large. However, in some particular cases, like lateral position in winter with opened windows, the time would be lower than an hour. Also, in the arm of driver, during summer season and travelling with completely opened windows, the erythema would appear in 40 min.

Finally, it has been seen that, with some exception (AP in May–June), time needed to appear a skin erythema is higher for the passenger than for driver as shown in Table 14.

Table 14 Time to erythema (in minutes) for driver position

Dosimeter position		February–March–April		May–June		July–August–September		October–November–December	
	Skin type	CW	OW	CW	OW	CW	OW	CW	OW
HD	I	443	1229	∞	774	2307	193	329	231
	II	554	1536	∞	968	2884	241	412	289
	III	775	2151	∞	1356	4037	337	576	405
	IV	997	2766	∞	1743	5190	434	741	520
	V	1219	3381	∞	2130	6344	530	906	636
	VI	1441	3995	∞	2518	7497	626	1071	752
LD	I	205	56	182	70	168	52	136	78
	II	256	70	228	88	210	65	170	97
	III	359	99	319	123	294	91	239	136
	IV	462	127	410	159	378	117	307	175
	V	564	156	501	194	461	143	375	214
	VI	667	184	592	229	545	169	443	253
VD	I	2680	800	∞	895	245	120	225	182
	II	3351	1000	∞	1119	306	151	281	227
	III	4691	1401	∞	1567	429	211	394	318
	IV	6031	1801	∞	2015	551	271	507	409
	V	7372	2201	∞	2463	673	331	619	499
	VI	8713	2601	∞	2911	796	391	732	590
AD	I	354	71	144	40	131	44	242	95
	II	442	88	180	50	164	55	303	118
	III	619	124	251	70	230	71	424	166
	IV	795	160	323	90	296	99	545	213
	V	972	196	395	110	361	121	666	260
	VI	1149	231	467	130	427	144	787	308

OW opened windows, *CW* closed windows

3.6 Discussion

Looking at the results, it can be concluded that solar height is the most important factor when measuring UV-R dose inside a vehicle. Thus, it has been shown that in months when solar height has a greater value (mainly winter months), there is a higher difference between dose received by the driver and by passenger. The reason is that the sun comes in more perpendicular on the driver position whereas the passenger is protected by the driver's shadow. That is because, for this particular study, the car position was always with driver's window pointed to sun.

In the case of months when solar height is lower (summer months), the effect of vehicle roof is to protect both the driver and passenger against radiation received.

References

Ambros-Rudolph, C. M., Hofmann, R., Richtig, E., Muller, M., Soyer, H. P., & Kerl, H. (2006). Malignant melanoma in marathon runners. *Archives of Dermatology, 1421*, 1471–1474.

Biosense Laboratories. Avaliable at http://www.biosense.de/home-e.htm

Bisset, D., Hannon, D., McBride, J., & Patrick, L. (1992). Photoaging of skin by UVA. In F. Urbach (Ed.), *Biological responses to ultraviolet a radiation* (pp. 181–188). Overland Park: Valdenmar Pblishing.

Blanca, V., Gurrea, G., Moreno, J. C., & Serrano, M. A. (2015). Maximum incident erythemally effective UV exposure received by construction workers, in Valencia, Spain. *Photochemistry and Photobiology, 91*, 1505–1509.

C.I.E. Commission Internationale de l'Eclairage. (1998). *Erythema reference action spectrum and standard erythema dose*. CIE S007E-1998. Vienna: CIE Central Bureau.

Engelsen, O., & Kylling, A. (2005). Fast simulation tool for ultraviolet radiation at the Earth's surface. *Optical Engineering, 44*(4), 041012. https://doi.org/10.1117/1.1885472. Available at http://nadir.nilu.no/

Furusawa, Y., Quintern, L. E., Holtschmidt, H., Koepke, P., & Saito, M. (1998). Determination of erythema-effective solar radiation in Japan and Germany with a spore monolayer film optimized fr the detection of UVA and UVA – Results of a field campaign. *Applied Microbiology and Biotechnology, 50*, 597–603.

Gurrea, G., Blanca, V., Moreno, J. C., & Serrano, M. A. (2018). Analysis of erythemal UVB dose received inside a car in Valencia, Spain. *Photochemistry and Photobiology, 94*, 390–397.

International Commission on Non-Ionizing Radiation Protection (ICNIRP). (2010). Protection of workers against ultraviolet radiation. *Health Physics, 99*(1), 66–87.

Lavker, R., & Kaidbey, K. (1997). The spectral dependence for UVA induced cumulative damage in human skin. *The Journal of Investigative Dermatology, 108*, 17–21.

Lavker, R. M., Gerberik, G. F., Veres, D., Irwin, C. J., & Kaidbey, K. H. (1995). Cummulative effects from repeated exposures to suberythemal doses of UVB and UVA in human skin. *The Journal of the American Academy of Dermatology, 32*, 53–62.

Lichte, V., Dennenmoser, B., Dietz, K., Hafner, H. M., Schlagenhauff, B., Garbe, C., Fischer, J., & Moehrle, M. (2010). Professional risk for skin cancer development in male mountain guides – A cross-sectional study. *JEADV, 24*, 797–804.

Lowe, N., Meyers, D., & Scott, I. (1995). Low doses of repetitive ultraviolet A induce morphologic changes in human skin. *The Journal of Investigative Dermatology, 105*, 739–743.

Moehrle, M., Dennenmoser, B., & Garbe, C. (2003). Continuous long-term monitoring of UV radiation in professional mountain guides reveals extremely high exposure. *International Journal of Cancer, 103*, 775–778.

Programa meteorología de la Fundación Centro de Estudios Ambientales del Mediterráneo (Generalitat Valenciana). Available at http://www.gva.es/ceamet/vigilancia/radUV/radUV.html

Richtig, E., Ambros-Rudoplh, C. M., Trapp, M., Lackner, J. K., Hofmann-Wellenhof, R., Kerl, H., & Schwaberger, G. (2008). Melanoma markers in marathon runners: Increase with sun exposure and physical strain. *Dermatology, 217*, 38–44.

Sayre, I., Cano, J., Picot, M., Meynadier, J., & Meunier, L. (1997). Immunosuppression induced by acute solar simulated ultraviolet exposure in humans: Prevention by a sunscreen with a sun protection factor of 15 and high UVA protection. *Journal of the American Academy of Dermatology, 37*, 187–194.

Serrano, M. A., Cañada, J., Moreno, J. C., & Gurrea, G. (2014). Personal UV exposure for different outdoor sports. *Photochemical & Photobiological Sciences, 13*, 671.

Vanicek, K., Frei, T., Litynska, Z., & Schmalweiser, A. (2000). *UV-index for the public*. Brussels: Office for official publications of the European communities.

Williams, H., Brett, J., & Du Vivier, A. (1989). Cyclist's melanoma. *Journal of the Royal College of Physicians of London, 23*(2), 114–115.

Monitoring the Genotoxic Potential of Sunlight and DNA Photoprotection of Sunscreen

James Eduardo Lago Londero and André Passaglia Schuch

Abstract

The effects of climate change and ozone depletion upon solar ultraviolet radiation (UV-R) incidence, as well as the risks of sunlight exposure to human health, need to be better understood. DNA molecule has been identified as the main cellular target of solar UV-R, and the UV-induced DNA damage is considered the initiating step of important biological process, such as the development skin cancers and aging. This chapter focuses on the use of physical and biological methods to measure the solar UV-R incidence and its genotoxic potential. The sunlight's DNA damage profiles induced at different latitudes are presented, and the use of DNA molecule as well as UV-hypersensitive human skin cells for the evaluation of sunscreen photoprotection is also discussed in the text.

Keywords

Sunscreen · Photoprotection · Genotoxic potential

1 Introduction

Ultraviolet radiation (UV-R) represents only 6.1% of the electromagnetic spectrum emitted by the sun, and it is divided in three wavebands: UVC (100–280 nm), UVB (280–315 nm), and UVA (315–400 nm). Nevertheless, only a small proportion of the solar UV-R reaches the terrestrial surface. Atmospheric oxygen and ozone completely absorb the UVC, and the ozone absorbs UVB photons. The UVA and UVB photons are the UV-R wavelengths biologically relevant for ecosystems and human health (Londero et al. 2017; Santos and Londero et al. 2018;

J. E. L. Londero · A. P. Schuch (✉)
Department of Biochemistry and Molecular Biology, Federal University of Santa Maria, Santa Maria, RS, Brazil

A. Dwivedi et al. (eds.), *Skin Aging & Cancer*,
https://doi.org/10.1007/978-981-13-2541-0_12

Schuch et al. 2017). The UVA photons correspond to approximately 95% and the UVB about 5% of the total solar UV-R wavelengths incident at the surface (Bais et al. 2015; Schuch et al. 2013).

Human exposure to sunlight can lead to beneficial or adverse effects. The main source of vitamin D occurs after exposure of skin to UVB photons. Vitamin D plays a determining role in the body maintenance of important processes, such as calcium homeostasis (Holick 2007). However, incident UVB and UVA rays are able to directly or indirectly damage biomolecules, such as DNA, proteins, and lipids, through energy transfer and electron relocations. Notably, DNA is the main target of the solar UV-R in living cells, and it is the molecule responsible for storing genetic information (Londero et al. 2019). Thus, UV-R can induce several types of DNA damage (or lesions) that can result in mutations or death in human skin cells. These cellular effects can lately result in carcinogenic processes or skin photoaging, respectively. Although UVB photons are more biologically effective than UVA photons, growing evidences suggest an important role of UVA in skin carcinogenesis, including formation of the cutaneous malignant melanoma, as well as in the skin photoaging (Schuch et al. 2017).

Therefore, an increasing knowledge about the incidence of solar UVB and UVA radiation at different latitudes becomes necessary for the accurate determination of their potential risks for human health (Bais et al. 2015; Schuch et al. 2013). In addition, the measurement of the DNA damage induced by solar UV-R in specific localities (Schuch et al. 2012a, 2013) would help to better characterize its genotoxic potential, as well as to develop more efficient sunscreens to protect people against both UVB and UVA rays (Schuch et al. 2012b, 2014). Thus, the information regarding ambient UV-R incidence and its biological effects are of vital interest for human health, especially in a scenario of increasing UV-R incidence (Bais et al. 2015).

2 Ambient UV-R Incidence

Several environmental factors can modulate the intensity of UV-R photons that reach the Earth's surface. The solar zenith angle (height of the sun above the horizon) consists of one of the main variables that influence the incidence of UV rays. Since solar zenith angle is modulated according to the latitude, hour of day, and season, the intensity of incident UV rays also varies according to these variables. Thus, UV-R is more intense when the sun is higher in the sky, which occurs in the tropics, near noon, and in the summer. Additionally, the pathway of sunlight through the atmosphere is longer at lower elevations, increasing the possibility of absorption of UV-R photons by the atmosphere, which results in a lower UV-R intensity at the surface compared with higher altitudes. In addition, the proportion of solar energy reflected from the surface (albedo) also modulates the amount of UV-R measured at a specific location (Schuch et al. 2017; McKenzie et al. 2007).

Latitudinal gradients are more decisive for the modulation of intensity of UVB than for the UVA rays. In South America, the UVB intensity in the city of Natal, Brazil (latitude 5°5′S), is 12-fold higher than in the city of Punta Arenas, Chile

(latitude 53°1′S). However, the UVA intensity is only twofold higher in Natal compared to Punta Arenas (Schuch et al. 2012a). This occurs mainly because UV-R rays travel a longer path through the atmosphere until they reach the higher latitudes in Earth, such as in Punta Arenas. Additionally, considering that UVB is more energetic than UVA, UVB is more prone to be absorbed by the atmosphere (mainly by ozone) than UVA (Schuch et al. 2017; McKenzie et al. 2007).

Other variations in solar UV-R incidence may occur due to changes in atmospheric constituents, which block the passage of the UV rays to the Earth's surface. Notably, cloud cover is a very important factor controlling UV-R incidence for any location on Earth. In addition, aerosols and trace gases, such as nitrogen dioxide and sulfur dioxide, are also capable to control solar UV-R in polluted urban areas. On the other hand, nitrogen dioxide and sulfur dioxide are able to scatter the UV-R, leading places shaded from direct sunlight to receive higher levels of reflected UV-R (Schuch et al. 2017; McKenzie et al. 2007).

When levels of clouds and aerosols are low or even null, the stratospheric ozone plays the leading role in absorbing solar UVB rays (Bais et al. 2015; McKenzie et al. 2007; Lucas et al. 2015). However, the constant and massive release of man-made chlorofluorocarbons (CFCs) has decreased ozone levels, since it catalyzes the breakdown of ozone to molecular oxygen. Consequently, a lower concentration of ozone directly results in an increase of UVB rays on Earth's surface. Recently, increases in ozone levels have been observed in different parts of the planet, since that global police measures have been taken in the last decades to reduce the release of ozone-depleting substances. However, an unexpected and persistent increase in global emissions of ozone-depleting trichlorofluoromethane (CFC-11) has recently been reported (Montzka et al. 2018). Furthermore, events of depletion of stratospheric ozone levels are still occurring in the winter and spring over the high latitudes (63–90°) of Southern and Northern hemispheres (WMO, Scientific Assessment of Ozone Depletion: 2014, 2015). Therefore, the consequences of climate changes for ozone recovery are unclear in the coming decades (Bais et al. 2015). It is important to note that the proportion of daily UVA radiation is naturally much higher than the UVB in any latitude, since that UVA rays hit the surface independently of the concentration of stratospheric ozone in the hemispheres (Schuch et al. 2017). Figure 1 shows the environmental factors that can alter the solar UV-R doses on Earth's surface.

3 Genotoxic Action of Ambient UV-R on Skin Cells

The main cellular effects induced by UV-R, cell death and mutagenesis, are direct biological consequences of DNA damage induction (Rastogi et al. 2010; Friedberg 2003). The UVC wavelengths are most effectively absorbed by DNA, followed by UVB and UVA. Since UVC does not reach the Earth's surface, UVB wavelengths are the main stressor emitted by sun for living beings. The incident UV-R wavelengths and the base composition of the DNA molecule determine the chemical nature and efficiency in the formation of DNA lesions. The direct excitation of the

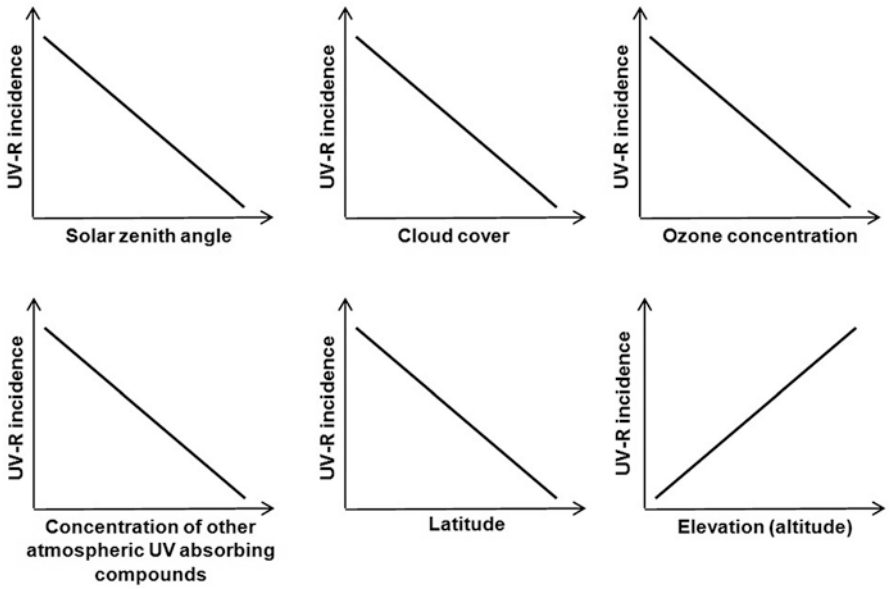

Fig. 1 Environmental factors that influence the incidence of solar UV-R on Earth's surface. Each graphic presents a tendency line between UV-R incidence with a specific environmental variable

DNA molecule by solar UVB and UVA wavelengths (mainly by UVB) triggers dimerization reactions between adjacent pyrimidines. These photoproducts, generically called as pyrimidine dimers, are lesions that distort the DNA molecule. The two main types of pyrimidine dimers are the cyclobutane pyrimidine dimers (CPDs) and the pyrimidine (6-4) pyrimidone photoproducts (6-4PPs). After further irradiation with wavelengths around 320 nm (UVA), the 6-4PP lesions can be converted to their Dewar valence isomers (Schuch et al. 2013; Rastogi et al. 2010; Friedberg 2003).

Solar UV-R can also damage DNA indirectly after photon absorption by other cellular chromophores and generation of reactive oxygen species (ROS), reactive nitrogen species (RNS), or reactive aldehydes. The DNA damage induced as consequence of redox imbalance (mostly oxidized guanine, such as the 7,8-dihydro-8-oxoguanine; 8-oxoG) occurs more effectively after UVA than UVB irradiation. Another type of indirect DNA lesion induced by UVB or UVA is the single-strand breaks (SSB), but this lesion has little involvement in the formation of mutations (Schuch et al. 2017; Friedberg 2003).

Throughout evolution cells have developed specific DNA repair mechanisms, which correct damaged DNA bases or small nucleotide sequences containing bulk lesions (Londero et al. 2019). These DNA repair systems are indispensable for the maintenance of genomic integrity and to avoid the cellular consequences of the permanence of DNA damage (Rastogi et al. 2010; Friedberg 2003). Two essential repair systems for the removal of UV-induced DNA damage in humans are the nucleotide excision repair (NER) and the base excision repair (BER) pathways.

Both perform DNA damage removal through processes that involves the coordinated participation of several proteins and multiple intermediate steps (Friedberg 2003). While NER is capable to repair the UV-induced pyrimidine dimers (Sancar and Tang 1993), BER is more prone to repair oxidized DNA bases (such as 8-oxoG), SSBs, and abasic sites (Wilson III and Bohr 2006). However, when the repair is inefficient, unrepaired DNA lesions may interfere in basic cellular processes, such as DNA replication and transcription, thereby leading to mutations and/or cell death (Schuch and Menck 2010).

The harmful effects of solar UV-R on skin cells are much more pronounced in the xeroderma pigmentosum (XP) patients. XP is an autosomal recessive syndrome mainly characterized by the hypersensitivity to sunlight. Most XP patients have mutations in one of the genes whose products are involved in the NER pathway. In addition, there is also the so-called XP variant, who has normal NER but is defective in the translesion synthesis (TLS) due to mutation in the *POLH* gene, which encodes the DNA polymerase η (Pol η) that performs the bypass of UV-induced pyrimidine dimers during DNA replication. As a consequence of the absence of the repair of UV-induced DNA lesions, XP patients show high frequency of skin cancer (Schuch et al. 2017; Friedberg 2003). This phenotype directly links the permanence of DNA lesion with the increase of mutation frequency and skin cancer development.

Despite the assurance that UVB is biologically more effective than UVA, it has been demonstrated that the participation of solar UVA radiation in skin carcinogenesis may be more drastic than previously assumed (Schuch et al. 2013, 2017). Interestingly, although the absorption of UVA by DNA is 4.2-fold lower than that of UVB radiation, the UVA incidence on Earth's surface is 20-fold higher than UVB (Schuch et al. 2017). Furthermore, the penetration capacity of UVA in the skin is higher than UVB, since UVA can reach melanocytes and dividing stem cells located in basal layers (Sage et al. 2012). It was demonstrated that UVA is able to induce mutation, chromosomal aberrations, and tumorigenic transformation in human skin cells (Wischermann et al. 2008). Consequently, the process of photocarcinogenesis can have important contribution of UVA wavelengths, although the mechanisms involved in this process are not completely known. Thus, it becomes necessary the development of more effective sunscreens against UVA wavelengths (Schuch et al. 2013, 2017).

Investigations concerning mutagenesis induced by UV-R demonstrate that the C–T transition is the mutation more frequently induced as response to pyrimidine dimers formed by both UVA and UVB (Pfeifer et al. 2005; Kappes et al. 2006; Ikehata et al. 2008). Interestingly, despite the higher induction of CPDs by equicytotoxic doses of UVB compared to UVA, pyrimidine dimers formed by UVA are considered more mutagenic than those induced by UVB. This is partly explained due to an ineffective cell cycle arrest, which leads to the progression of DNA replication in the presence of DNA lesions and the accumulation of mutations (Runger et al. 2012). On the other hand, when mouse skin is exposed to UVA wavelengths, it is observed that only 6% of the mutation spectrum is related to the formation of 8-oxoG, the main oxidized DNA damage (Ikehata et al. 2008). It is suggested that CPDs have a higher contribution to the sunlight-induced mutagenesis in contrast

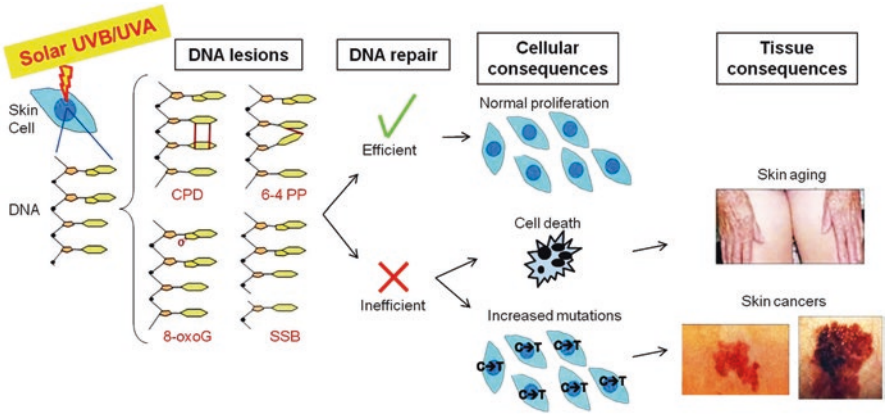

Fig. 2 Genotoxic action of UV-R on skin cells and its consequences for human health

with 8-oxoG or even 6-4PPs. This could be explained by the rapid efficient removal of 8-oxoG and 6-4PPs by the action of BER and NER enzymes, respectively (Sage et al. 2012; Besaratinia et al. 2008). A representative scheme of the genotoxic action of solar UV-R is shown in Fig. 2.

Despite the suggested little involvement of UV-induced oxidative DNA damage in the mutagenic processes induced by sunlight, the induction of redox imbalance (mainly by UVA) requires attention, because reactive aldehydes, ROS, and RNS may damage other cell structures and molecules than DNA itself (Schuch et al. 2017). For instance, oxidized proteins may cause cross-links with DNA blocking its metabolism processes, such as replication and transcription (Girard et al. 2008; Hoerter et al. 2008; Davies 2016; Radman 2016; Graindorge et al. 2015). Additionally, the lipid peroxidation process can oxidize the cell membranes destabilizing them. Hence, when these events are not lethal to the cells, they can lead to unsaturated aldehydes, which may form mutagenic adducts in DNA (Blair 2008; Medeiros 2009).

4 Physical and Biological Methods for Monitoring the Ambient UV-R

Accurate and reliable physical and biological methods are needed for monitoring of solar UV-R incidence and to access the risks to human health (Schuch et al. 2017). Broadband detectors and spectroradiometers are the most common tools for monitoring of solar UV-R. Broadband detectors are capable to measure a certain UV wavelength range and use special filters to measure UVB or UVA wavebands. Spectroradiometers are sensitive to solar UV-R at individual wavelengths (Godar 2005).

Examples of broadband detectors that take measurements at 280–315 nm (UVB waveband) and 315–400 nm (UVA waveband) are specific radiometers from EKO

Instruments Trading, Tokyo, Tokyo-to, Japan. Furthermore, other example of broadband detectors is Robertson-Berger sunburn meter (RB meter), which has a spectral sensitivity that is similar to the human erythemal response, thereby measuring only the "burning" solar UVB rays (Godar 2005). On the other hand, the Brewer equipment is an example of spectroradiometer (Kipp and Zonen Instruments, Inc., Saskatoon, Saskatchewan, Canada), which measures the full sky and spectrally resolves UV-R between 286.5 and 363 nm through 0.5 nm wavelength increments. In addition, this instrument also infers the total column of ozone in the atmosphere (in Dobson units). However, this type of equipment has disadvantages that make their use difficult for routine measurements, which include high cost, difficulty of operation, requirement of constant calibration, and complementation with biological models (Godar 2005).

Another approach for the determination of the biologically effective dose of UV-R that reaches the terrestrial surface involves the use of biological systems to measure specific photobiological responses. In general, a biosensor integrates the UV spectral response and the photobiological effect that is measured. Notably, some characteristics must be considered in the elaboration of new biosensors, such as high resistance against changing environmental conditions, suitability for routine measurement, and representativeness of a possible risk or benefit to human health or ecosystems. Furthermore, it is important to note that the spectral response (UVB/UVA) should be in agreement with a specific photobiological process and that the quantification of the biological effects of UV-R should be undertaken in measurable units (Schuch et al. 2013).

The use of organisms to evaluate the genotoxic potential of sunlight on ecosystems is not a simple task (Londero et al. 2019). Over the last decades, various simple systems have been developed to be used as biological UV dosimeters, such as provitamin D3 (Galkin and Terenetskaya 1999), uracil thin layers (Gróf et al. 1996; Horvath et al. 2001), DNA (Schuch et al. 2009; Yoshida and Regan 1997a; Regan and Yoshida 1995; George et al. 2002) or different bacteriophages (Yoshida and Regan 1997b; Hegedus et al. 2003), spores from *Bacillus subtilis* (Munakata et al. 1998, 2001, 2006; Berces et al. 1999), and eukaryotic cells in culture (Schuch et al. 2014; Rettberg et al. 1999). Most of these systems measure directly or indirectly the DNA damage induced by solar UV-R.

An example of very simple, portable, and robust DNA dosimeter is based on minidots of purified and dried bacteriophage λ DNA placed on an UV-R transparent biofilm (Yoshida and Regan 1997a, b; Regan and Yoshida 1995). Another example of DNA dosimeter based on the use of bacteriophage DNA is the phage T7 dosimeter. In this case, the lesions are measured through different DNA fragments using a quantitative polymerase chain reaction methodology (Hegedus et al. 2003; Rontó et al. 1992, 1994). In both dosimeter assays, the pyrimidine dimers block in vitro DNA replication by Taq DNA polymerase, thereby decreasing the amplification of a damaged DNA segment. Nonetheless, 6-4PPs are considered more effective in blocking replication than the CPDs, due to the greater structural distortion formed by this type of lesion in the DNA molecule. Additionally, the use of bacteriophage systems allows the evaluation of other biological consequences related to DNA damage induction, such as the inactivation

(killing) of a phage particle. However, it is important to consider that caution should be taken with the data obtained through the use of dried DNA used in these systems, since it has been demonstrated that pyrimidine dimers are induced at a much lower frequency in the absence of water than in a water solution (Schuch et al. 2013).

Aqueous solution of naked DNA is also used in DNA dosimeters. In one of such system, a naked calf thymus DNA solution is maintained in cylindrical quartz tubes, and the CPDs are identified by using a specific antibody against this lesion (George et al. 2002). Another DNA dosimeter that also uses naked calf thymus DNA solution in quartz tubes was complemented with the bacteriophage PWH3a-P1, which infects the bacterium *Vibrio natriegens*, allowing for the measurement of DNA damage and loss of infectivity of the bacteriophage after sun exposure (Wilhelm et al. 2002).

In addition, a suitable, reliable, low cost, resistant, highly UV transparent DNA dosimeter system uses a purified plasmid DNA solution to artificial UV lamps and sunlight (Schuch et al. 2009, 2013). This DNA dosimeter allows the calculation of the biologically effective dose (BED) of UV-R by the quantification of the amount of different types of DNA lesions generated after an exposure period. In this in vitro methodology, DNA lesions are detected either directly (by DNA breaks) or by using lesion-specific DNA repair enzymes (such as T4 bacteriophage endonuclease V, yeast ultraviolet damage endonuclease, and the *E. coli* formamidopyrimidine-DNA glycosylase) that cleave DNA at the damage site. Quantification of the number of lesions is performed by densitometric analyses after separation of supercoiled and circular relaxed plasmid DNA forms through electrophoresis migration in agarose gel. The amount of each type of DNA lesion is represented as the average number of enzyme-sensitive sites per kilobase pair (ESS/kbp). Therefore, this DNA dosimeter proves to be an efficient tool that allows the detection of several types of UV-induced DNA damage, such as CPDs, 6-4PPs, and oxidized DNA bases (Schuch et al. 2009). The use of this biological system made it possible to verify the DNA damage profiles induced by sunlight at different latitudes in South America and to evaluate DNA photoprotection of sunscreen, as described below (Schuch et al. 2012a, b).

The application of biosensors in combination with other physical and/or meteorological tools helps to improve knowledge regarding the interactions between climate changes, ozone depletion, and increased solar UV-R doses, as well as their impact on biomolecules, human health, and ecosystems (Yagura et al. 2011). Figure 3 presents examples of UV-R radiometers and the DNA-dosimeter system for monitoring the incidence solar UV-R and its genotoxic potential.

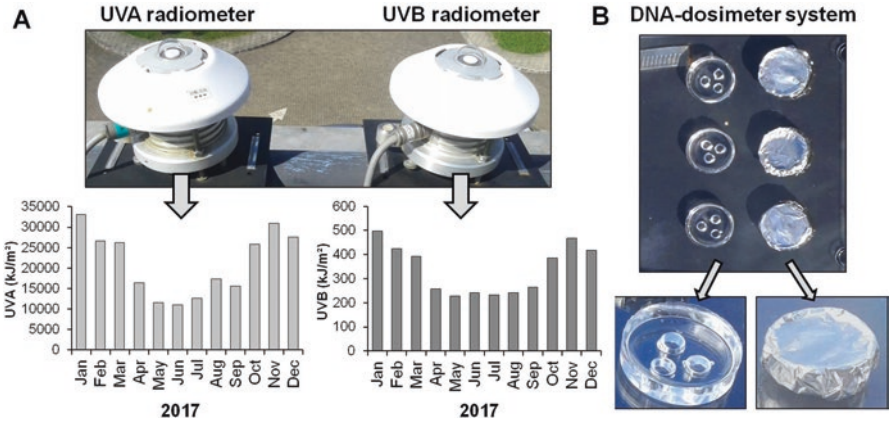

Fig. 3 Examples of UVA and UVB radiometers and the use of DNA-dosimeter system to evaluate the genotoxic potential of sunlight. (**a**) UVA radiometer MS-212A (315–400 nm) and UVB radiometer MS-212 W (280–315 nm) from EKO Instruments Trading Co., Ltd. installed at the Federal University of Santa Maria, Brazil. These radiometers are continuously measuring the daily incidence of solar UV-R in the city of Santa Maria, RS, Brazil (latitude 29°S). Below, it is shown the result of the UVA and UVB doses that reached the surface at this latitude throughout the months of the year 2017 (cumulative monthly doses in kJ/m^2). (**b**) DNA-dosimeter system used for the exposures of DNA samples to sunlight. DNA-dosimeter system covered with an aluminum foil represents the nonirradiated control samples for comparative analysis with the exposed DNA samples (non-covered system)

5 Sunlight's DNA Damage Profiles at Different Latitudes

To monitor the incident solar UV-R and its genotoxic potential, field experiments were performed with the DNA-dosimeter system in parallel with measurements of solar UVB/UVA incidence at different latitudes in South America (Schuch et al. 2012a). The obtained data demonstrates that the incidence of UVB radiation increased according to the decrease of latitude. For instance, UVB doses reached 1.9-, 5.3-, and 12.1-fold higher in São Martinho da Serra (29°4′S), São Paulo (23°3′S), and Natal (5°5′S), respectively, in comparison with Punta Arenas (53°1′S). On the other hand, UVA doses were about twofold higher in Natal compared to Punta Arenas. Interestingly, any decrease in the latitude is accompanied by a corresponding increase in the formation of pyrimidine dimers in relation to oxidized DNA bases. On the other hand, an increase in latitude favors the induction of both oxidized DNA bases and SSBs, followed by a decrease in the formation of 6-4PPs. Notably, although the amount of CPD lesions varied along the latitudinal gradient, their induction profiles were similar in the four locations. Therefore, 6-4PP lesions can be considered as a biomolecular marker for the amount of incidental solar UVB radiation and oxidized DNA bases for incidental UVA. Thus, the genotoxic potential of sunlight does vary according to the latitude (Schuch et al. 2012a). Figure 4 represents the DNA damage profiles induced by sunlight at different latitudes of South America.

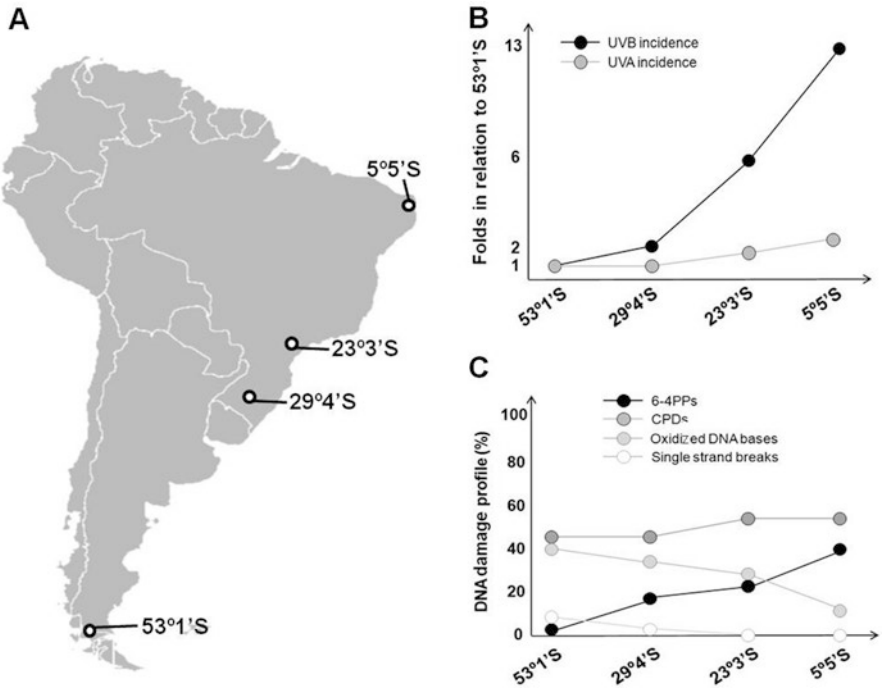

Fig. 4 Sunlight's DNA damage profiles induced at different latitudes. (**a**) Latitudes in South America where the DNA damage profiles were determined. (**b**) Fold change of solar UVB and UVA doses measured at each latitude in relation to the 53°1′S. (**c**) Representative description of the variations in DNA damage profiles at different latitudes in South America

Therefore, this figure shows clearly that the incidence of UVB radiation is higher in low latitudes, where the levels of stratospheric ozone are naturally lower, resulting in higher levels of 6-4PPs. On the other hand, the UVA incidence on Earth's surface does not vary drastically as UVB incidence throughout South America (Schuch et al. 2012a, 2013). This information is extremely relevant for guiding future works aiming at indicating the molecular impact of variations in solar UV incidence resulting from variations in stratospheric ozone, climate changes, or both, as well as for the development of more effective sunscreen.

6 Evaluation of DNA Photoprotection of Sunscreen

The interaction between ozone depletion and global warming may increase the incidence of skin cancer in the UK from 5000 to 6000 cases per year by 2050 (Diffey 2004). Worryingly, populations of tropical countries, such as Brazil, may suffer higher consequences, since exposure to very high UV-R doses becomes more frequent. Furthermore, this scenario is even more dramatic for people suffering from diseases that are associated with extreme sensitivity to solar UV-R, such as xeroderma pigmentosum (XP) (Munford et al. 2017). Therefore, photoprotection has

become a topic of increasing relevance in public healthcare, and the use of sunscreen became one of the main preventive measures against the deleterious effects of solar UV-R (Schuch et al. 2014).

The protective efficiency of sunscreens has been evaluated by measuring the induction of erythema in human skin, which is expressed as the sun protection factor (SPF), by the in vivo persistent pigment darkening (PPD), as well as by the in vitro UVA-PF. However, none of these methodologies can evaluate DNA photoprotection provided by sunscreen formulations. DNA photoprotection has been demonstrated using skin of human explants, mice, reconstructed human skin models, and in vitro cultured human cells (Schuch et al. 2012b, 2013).

In the last years, the use of a DNA-dosimeter system has been used to characterize the DNA photoprotection of sunscreen formulations (Schuch et al. 2012b). The sun protection factor for DNA (DNA-SPF) is measured by the ratio between the total amount of DNA lesions (CPDs + oxidized DNA bases) induced by UV radiation in plasmid DNA samples without sunscreen and the total amount of DNA damage verified in irradiated samples in the presence of sunscreen. This biological dosimeter also provides the calculation of the percentage of DNA photoprotection (percentage of protection against the induction of both CPDs and oxidized DNA bases). It provides a simple and clear manner to qualify the biological protection of a specific formulation. In general, the sunscreen provides good protection against DNA lesion induced by UVB radiation. However, the same sunscreen is less efficient in protecting against the formation of oxidized DNA bases and CPDs induced by UVA, suggesting that improvements are necessary to increase protection against UVA wavelengths (Schuch et al. 2012b). Figure 5 shows an example of the evaluation of DNA photoprotection of sunscreen through the use of DNA-system dosimeters.

Until recently, there was none elaborated methodology to verify the photoprotection efficiency of sunscreens based on the needs of people with hypersensitivity to sunlight, such as the patients with XP syndrome. Then, a DNA-based cell dosimeter was designed to measure the protective properties of sunscreen directly in human skin fibroblasts obtained from XP patients (Schuch et al. 2014). This biosensor enables the exposure of human cultured cells to sunlight in a hermetically sealed, sterile, and highly UV-R transparent environment. After irradiation, this cell dosimeter quantifies the sun protection factor for lethal damage (LD-SPF), measuring cell viability after UV treatment, as well as the sun protection factor for genomic DNA (genomic DNA-SPF), using a CPD-specific antibody (Schuch et al. 2014).

Through the use of DNA-based cell dosimeter four commercial sunscreens with different labeled SPFs (8, 15, 35, and 60; same brand) were tested for their ability to protect XP cells against UVB, UVA radiation, and natural sunlight. The tested sunscreen presented efficient photoprotection against the induction of DNA damage and mortality after UVB exposure. However, the same protective effect was not observed for UVA radiation and natural sunlight. This data emphasizes the low protective efficiency of the tested commercial sunscreen for UVA wavelengths of sunlight. Therefore, it is necessary to improve the efficiency of photoprotection provided by sunscreen lotions against UVA wavelengths, as well as improve the methodologies available for cosmetic industry to determine photoprotection of

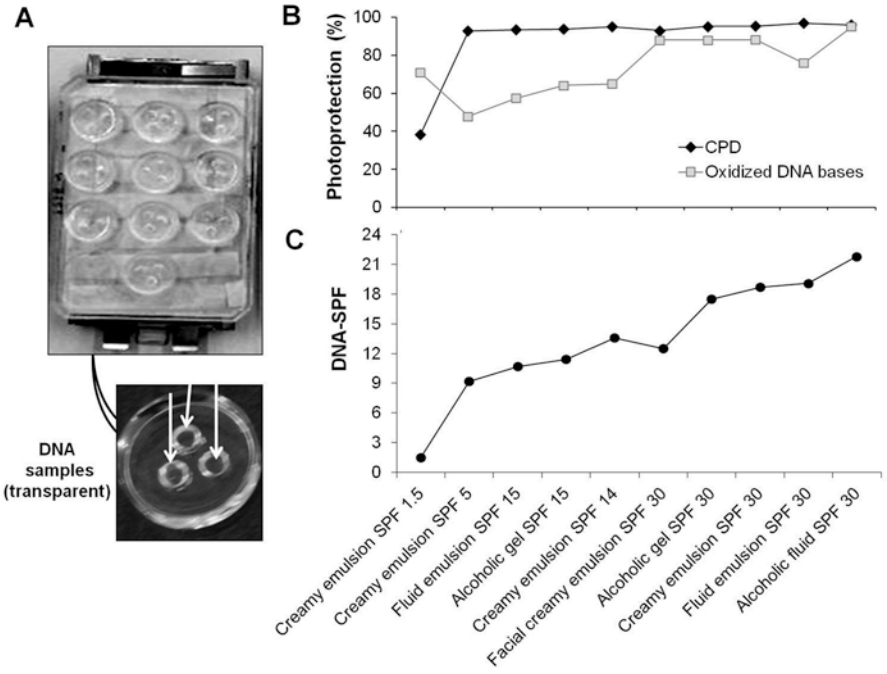

Fig. 5 Evaluation of DNA photoprotection of sunscreen. (**a**) Exposure of DNA dosimeters to a solar simulator for the verification of DNA photoprotection efficiency of different sunscreens. (**b**) Percentages of protection against CPDs and oxidized DNA bases of several products containing sunscreen. (**c**) Sun protection factor for DNA (DNA-SPF) of several sunscreen formulations

sunscreen in order to improve the efficiency of sunscreen for the use by the general population (Schuch et al. 2012b, 2014).

7 Concluding Remarks

Solar UV-R is an important environmental genotoxic agent that a considerable part of the world's population is exposed daily. Worryingly, DNA damage induced by UV-R is considered the main cause of skin aging and skin cancer development, including the malignant melanoma. However, the understanding of how the solar UV wavelengths act in the skin cancer and aging is still not complete. Therefore, accurate and reliable physical and biological dosimeters are needed for the monitoring of solar UV-R exposure and to access the risks to human health. The parallel application of physical methods with DNA-based dosimeters offers the possibility to develop works directly in the environment with different objectives, such as (1) to quantify the DNA lesions induced by sunlight, (2) to evaluate the changes in the

DNA damage profiles at different latitudes and correlate it with the incidence of UVA and UVB wavelengths, and (3) to evaluate the efficiency of DNA photoprotection provided by commercial sunscreen.

References

Bais, A. F., McKenzie, R. L., Bernhard, G., Aucamp, P. J., Ilyas, M., Madronich, S., & Tourpali, K. (2015). Ozone depletion and climate change: Impacts on UV radiation. *Photochemical & Photobiological Sciences, 14*, 19–52.

Berces, A., Fekete, A., Gaspar, S., Gróf, P., Rettberg, P., Horneck, G., & Rontó, G. (1999). Biological UV dosimeters in the assessment of the biological hazard from environmental radiation. *Journal of Photochemistry and Photobiology. B, 53*, 36–43.

Besaratinia, A., Kim, S. I., & Pfeifer, G. P. (2008). Rapid repair of UVA-induced oxidized purines and persistence of UVB-induced dipyrimidine lesions determine the mutagenicity of sunlight in mouse cells. *The FASEB Journal, 22*, 2379–2392.

Blair, I. A. (2008). DNA adducts with lipid peroxidation products. *The Journal of Biological Chemistry, 283*, 15545–15549.

Davies, M. J. (2016). Protein oxidation and peroxidation. *The Biochemical Journal, 473*, 805–825.

Diffey, B. (2004). Climate change, ozone depletion and the impact on ultraviolet exposure of human skin. *Physics in Medicine and Biology, 49*, R1–R11.

dos Santos, C. P., Londero, J. E. L., dos Santos, M. B., Feltrin, R. d. S., Loebens, L., Moura, L. B., Cechin, S. Z., & Schuch, A. P. (2018). Sunlight-induced genotoxicity and damage in keratin structures decrease tadpole performance. *Journal of Photochemistry and Photobiology B: Biology, 181*, 134–142.

Friedberg, E. C. (2003). DNA damage and repair. *Nature, 421*, 436–440.

Galkin, O. N., & Terenetskaya, I. P. (1999). 'Vitamin D' biodosimeter: Basic characteristics and potential applications. *Journal of Photochemistry and Photobiology. B, 53*, 12–19.

George, A. L., Peat, H. J., & Buma, A. G. (2002). Evaluation of DNA dosimetry to assess ozone-mediated variability of biologically harmful radiation in Antarctica. *Photochemistry and Photobiology, 76*, 274–280.

Girard, P. M., Pozzebon, M., Delacote, F., Douki, T., Smirnova, V., & Sage, E. (2008). Inhibition of S-phase progression triggered by UVA-induced ROS does not require a functional DNA damage checkpoint response in mammalian cells. *DNA Repair, 7*, 1500–1516.

Godar, D. E. (2005). UV doses worldwide. *Photochemistry and Photobiology, 81*, 736–749.

Graindorge, D., Martineau, S., Machon, C., Arnoux, P., Guitton, J., Francesconi, S., Frochot, C., Sage, E., & Girard, P. M. (2015). Singlet oxygen-mediated oxidation during UVA radiation alters the dynamic of genomic DNA replication. *PLoS One, 10*, e0140645.

Gróf, P., Gáspár, S., & Rontó, G. (1996). Use of uracil thin layer for measuring biologically effective UV dose. *Photochemistry and Photobiology, 64*, 800–806.

Hegedus, M., Modos, K., Rontó, G., & Fekete, A. (2003). Validation of phage T7 biological dosimeter by quantitative polymerase chain reaction using short and long segments of phage T7 DNA. *Photochemistry and Photobiology, 78*, 213–219.

Hoerter, J. D., Ward, C. S., Bale, K. D., Gizachew, A. N., Graham, R., Reynolds, J., Ward, M. E., Choi, C., Kagabo, J. L., Sauer, M., Kuipers, T., Hotchkiss, T., Banner, N., Chellson, R. A., Ohaeri, T., Gant, L., & Vanderhill, L. (2008). Effect of UVA fluence rate on indicators of oxidative stress in human dermal fibroblasts. *International Journal of Biological Sciences, 4*, 63–70.

Holick, M. F. (2007). Vitamin D deficiency. *The New England Journal of Medicine, 357*, 266–281.

Horvath, R., Kerekgyarto, T., Csucs, G., Gaspar, S., Illyes, P., Rontó, G., & Papp, E. (2001). The effect of UV irradiation on uracil thin layer measured by optical waveguide light mode spectroscopy. *Biosensors & Bioelectronics, 16*, 17–21.

Ikehata, H., Kawai, K., Komura, J., Sakatsume, K., Wang, L., Imai, M., Higashi, S., Nikaido, O., Yamamoto, K., Hieda, K., Watanabe, M., Kasai, H., & Ono, T. (2008). UVA1 genotoxicity is

mediated not by oxidative damage but by cyclobutane pyrimidine dimers in normal mouse skin. *The Journal of Investigative Dermatology, 128*, 2289–2296.

Kappes, U. P., Luo, D., Potter, M., Schulmeister, K., & Runger, T. M. (2006). Short- and long-wave UV light (UVB and UVA) induce similar mutations in human skin cells. *The Journal of Investigative Dermatology, 126*, 667–675.

Londero, J. E. L., dos Santos, M. B., & Schuch, A. P. (2019). Impact of solar UV radiation on amphibians: Focus on genotoxic stress. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 842*, 14–21.

Londero, J. E. L., dos Santos, C. P., Segatto, A. L. A., & Passaglia Schuch, A. (2017). Impacts of UVB radiation on food consumption of forest specialist tadpoles. *Ecotoxicology and Environmental Safety, 143*, 12–18.

Lucas, R. M., Norval, M., Neale, R. E., Young, A. R., de Gruijl, F. R., Takizawa, Y., & van der Leun, J. C. (2015). The consequences for human health of stratospheric ozone depletion in association with other environmental factors. *Photochemical & Photobiological Sciences, 14*, 53–87.

McKenzie, R. L., Aucamp, P. J., Bais, A. F., Bjorn, L. O., & Ilyas, M. (2007). Changes in biologically-active ultraviolet radiation reaching the Earth's surface. *Photochemical & Photobiological Sciences, 6*, 218–231.

Medeiros, M. H. G. (2009). Exocyclic DNA adducts as biomarkers of lipid oxidation and predictors of disease. Challenges in developing sensitive and specific methods for clinical studies. *Chemical Research in Toxicology, 22*, 419–425.

Montzka, S. A., Dutton, G. S., Yu, P., Ray, E., Portmann, R. W., Daniel, J. S., Kuijpers, L., Hall, B. D., Mondeel, D., Siso, C., Nance, J. D., Rigby, M., Manning, A. J., Hu, L., Moore, F., Miller, B. R., & Elkins, J. W. (2018). An unexpected and persistent increase in global emissions of ozone-depleting CFC-11. *Nature, 557*(7705), 413–417.

Munakata, N., Ono, M., & Watanabe, S. (1998). Monitoring of solar-UV exposure among school-children in five Japanese cities using spore dosimeter and UV-coloring labels. *Japanese Journal of Cancer Research, 89*, 235–245.

Munakata, N., Makita, K., Bolsée, D., Gillotay, D., & Horneck, G. (2001). Spore dosimetry of solar UV radiation: Applications to monitoring of daily irradiance and personal exposure. *Advances in Space Research, 26*, 1995–2003.

Munakata, N., Cornain, S., Kanoko, M., Mulyadi, K., Lestari, S., Wirohadidjojo, W., Bolsee, D., Kazadzis, S., Meyer-Rochow, V., Schuch, N., Casiccia, C., Kaneko, M., Liu, C. M., Jimbow, K., Saida, T., Nishigori, C., Ogata, K., Inafuku, K., Hieda, K., & Ichihashi, M. (2006). Biological monitoring of solar UV radiation at 17 sites in Asia, Europe and South America from 1999 to 2004. *Photochemistry and Photobiology, 82*, 689–694.

Munford, V., Castro, L. P., Souto, R., Lerner, L. K., Vilar, J. B., Quayle, C., Asif, H., Schuch, A. P., de Souza, T. A., Ienne, S., Alves, F. I. A., Moura, L. M. S., Galante, P. A. F., Camargo, A. A., Liboredo, R., Pena, S. D. J., Sarasin, A., Chaibub, S. C., & Menck, C. F. M. (2017). A genetic cluster of patients with variant xeroderma pigmentosum with two different founder mutations. *The British Journal of Dermatology, 176*, 1270–1278.

Pfeifer, G. P., You, Y. H., & Besaratinia, A. (2005). Mutations induced by ultraviolet light. *Mutation Research, 571*, 19–31.

Radman, M. (2016). Protein damage, radiation sensitivity and aging. *DNA Repair, 44*, 186–192.

Rastogi, R. P., Richa, A., Kumar, M. B., & Tyagi, R. P. S. (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *Journal of Nucleic Acids, 2010*, 592980.

Regan, J. D., & Yoshida, H. (1995). DNA UVB dosimeters. *Journal of Photochemistry and Photobiology B: Biology, 31*, 57–61.

Rettberg, P., Horneck, G., Baumstark-Khan, C., & Amanatidis, G. T. (1999). *Biological UV dosimetry, a tool for assessing the impact of UV radiation on health and ecossystems*. Brussels: European Commission.

Rontó, G., Gáspár, S., & Berces, A. (1992). Phage T7 in biological UV dose measurement. *Journal of Photochemistry and Photobiology. B, 12*, 285–294.

Rontó, G., Gáspár, S., & Gugolya, Z. (1994). Ultraviolet dosimetry in outdoor measurements based on bacteriophage T7 as a biosensor. *Photochemistry and Photobiology, 59*, 209–214.

Runger, T. M., Farahvash, B., Hatvani, Z., & Rees, A. (2012). Comparison of DNA damage responses following equimutagenic doses of UVA and UVB: A less effective cell cycle arrest with UVA may render UVA-induced pyrimidine dimers more mutagenic than UVB-induced ones. *Photochemical & Photobiological Sciences, 11*, 207–215.

Sage, E., Girard, P. M., & Francesconi, S. (2012). Unravelling UVA-induced mutagenesis. *Photochemical & Photobiological Sciences, 11*, 74–80.

Sancar, A., & Tang, M.-S. (1993). Nucleotide excision repair. *Photochemistry and Photobiology, 57*, 905–921.

Schuch, A. P., & Menck, C. F. M. (2010). The genotoxic effects of DNA lesions induced by artificial UV-radiation and sunlight. *Journal of Photochemistry and Photobiology. B, 99*, 111–116.

Schuch, A. P., Galhardo, R. S., Lima-Bessa, K. M., Schuch, N. J., & Menck, C. F. (2009). Development of a DNA-dosimeter system for monitoring the effects of solar-ultraviolet radiation. *Photochemical & Photobiological Sciences, 8*, 111–120.

Schuch, A. P., Yagura, T., Makita, K., Yamamoto, H., Schuch, N. J., Agnez-Lima, L. F., MacMahon, R. M., & Menck, C. F. (2012a). DNA damage profiles induced by sunlight at different latitudes. *Environmental and Molecular Mutagenesis, 53*, 198–206.

Schuch, A. P., Lago, J. C., Yagura, T., & Menck, C. F. (2012b). DNA dosimetry assessment for sunscreen genotoxic photoprotection. *PLoS One, 7*, e40344.

Schuch, A. P., Garcia, C. C., Makita, K., & Menck, C. F. (2013). DNA damage as a biological sensor for environmental sunlight. *Photochemical & Photobiological Sciences, 12*, 1259–1272.

Schuch, A. P., Moraes, M. C. S., Yagura, T., & Menck, C. F. M. (2014). Highly sensitive biological assay for determining the photoprotective efficacy of sunscreen. *Environmental Science & Technology, 48*, 11584–11590.

Schuch, A. P., Moreno, N. C., Schuch, N. J., Menck, C. F. M., & Garcia, C. C. M. (2017). Sunlight damage to cellular DNA: Focus on oxidatively generated lesions. *Free Radical Biology & Medicine, 107*, 110–124.

Wilhelm, S. W., Jeffrey, W. H., Suttle, C. A., & Mitchell, D. L. (2002). Estimation of biologically damaging UV levels in marine surface waters with DNA and viral dosimeters. *Photochemistry and Photobiology, 76*, 268–273.

Wilson, D. M., III, & Bohr, V. A. (2006). The mechanics of base excision repair, and its relationship to aging and disease. *DNA Repair, 6*, 544–559.

Wischermann, K., Popp, S., Moshir, S., Scharfetter-Kochanek, K., Wlaschek, M., de Gruijl, F., Hartschuh, W., Greinert, R., Volkmer, B., Faust, A., Rapp, A., Schmezer, P., & Boukamp, P. (2008). UVA radiation causes DNA strand breaks, chromosomal aberrations and tumorigenic transformation in HaCaT skin keratinocytes. *Oncogene, 27*, 4269–4280.

WMO, Scientific Assessment of Ozone Depletion: 2014. (2015). *Global ozone research and monitoring project*. Geneva: WMO (World Meteorological Organization).

Yagura, T., Makita, K., Yamamoto, H., Menck, C. F., & Schuch, A. P. (2011). Biological sensors for solar ultraviolet radiation. *Sensors, 11*, 4277–4294.

Yoshida, H., & Regan, J. D. (1997a). UVB DNA dosimeters analyzed by polymerase chain reactions. *Photochemistry and Photobiology, 66*, 82–88.

Yoshida, H., & Regan, J. D. (1997b). Solar UVB dosimetry by amplification of short and long segments in phage lambda DNA. *Photochemistry and Photobiology, 66*, 672–675.