

# Contents

<b>1. Introduction</b>	<b>5</b>
1.1. The mammalian skin	5
1.1.1. Skin architecture and differentiation	5
1.1.2. Skin function	7
1.2. Ultraviolet radiation (UVR)	7
1.2.1. Photobiology of solar UVR	7
1.2.2. UVR defense system	8
1.3. Reactive oxygen species (ROS) and the antioxidant defense system	9
1.3.1. ROS and oxidative stress	9
1.3.2. Antioxidant defense system and glutathione	10
1.4. NF-E2-related factor (Nrf) transcription factors	12
1.4.1. Domain composition of the Nrf transcription factors	12
1.4.2. Expression and function of the Nrf family members	12
1.4.3. Regulation of the Nrf transcription factors	14
1.4.4. Role of the Nrf transcription factors in murine skin	15
1.5. micro (mi) RNAs	17
1.5.1. Brief history of RNAi and miRNA	17
1.5.2. miRNA biogenesis and mechanisms of action	18
1.5.3. miRNAs in the skin	21
1.5.4. Background information on miR-21	22
1.6. Aim of the PhD thesis	22
<b>2. Materials</b>	<b>25</b>
2.1. Chemicals and other consumables	25
2.2. Enzymes	28
2.3. Buffers	28

2.4. Antibodies . . . . .	29
2.4.1. Antibodies for immunostaining . . . . .	29
2.4.2. Antibodies for flow cytometry . . . . .	30
2.5. Cell culture reagents . . . . .	30
2.6. Primers . . . . .	31
2.7. Kits . . . . .	33
2.8. Standard buffers and solutions . . . . .	34
<b>3. Methods</b>	<b>37</b>
3.1. General . . . . .	37
3.1.1. Agarose gel electrophoresis and nucleic acid visualization . . . . .	37
3.1.2. Determination of RNA and DNA concentrations . . . . .	37
3.2. RNA Methods . . . . .	38
3.2.1. General directions for working with RNA . . . . .	38
3.2.2. RNA isolation using GSCN . . . . .	38
3.2.3. RNase Protection Assay (RPA) . . . . .	39
3.3. DNA Methods . . . . .	42
3.3.1. Genotyping . . . . .	42
3.3.2. Gene expression analysis – mRNA quantification . . . . .	44
3.3.3. miRNA-expression analysis . . . . .	46
3.4. Protein methods . . . . .	47
3.4.1. Preparation of protein lysates . . . . .	47
3.4.2. SDS PAGE . . . . .	48
3.4.3. Western blot . . . . .	48
3.4.4. Determination of epidermal glutathione levels . . . . .	50
3.5. Cell culture . . . . .	50
3.5.1. Cultivation of primary human foreskin keratinocytes (HFK) . . . . .	50
3.5.2. Cultivation of immortalized primary mouse keratinocytes . . . . .	50
3.5.3. Isolation of primary murine keratinocytes . . . . .	51
3.5.4. Treatment of primary mouse keratinocytes with glucose oxidase, H <sub>2</sub> O <sub>2</sub> or menadione . . . . .	52
3.5.5. Irradiation of primary mouse keratinocytes with UVA or UVB . . . . .	52
3.5.6. Immunofluorescence analysis of cultured cells . . . . .	52
3.5.7. TUNEL staining of cultured cells . . . . .	52

3.5.8. MTT assay . . . . .	53
3.5.9. Human placental alkaline phosphatase (hPAP) histochemistry of cultured cells . . . . .	53
3.5.10. Flow cytometric methods . . . . .	53
3.5.11. Transfection of cultured cells . . . . .	54
3.6. FACS and MACS . . . . .	55
3.6.1. Preparation of epidermal single cell suspensions . . . . .	55
3.6.2. Analysis of epidermal single cell suspensions by flow cytometry and FACS . . . . .	55
3.6.3. Magnetic associated cells sorting (MACS) of Langerhans cells . . . . .	56
3.7. Histology . . . . .	56
3.7.1. Acidic ethanol-/PFA-fixed paraffin sections . . . . .	57
3.7.2. Cryosections . . . . .	57
3.7.3. Staining of tissue sections . . . . .	57
3.8. Animal experiments . . . . .	62
3.8.1. Mouse strains . . . . .	62
3.8.2. UV irradiation . . . . .	62
3.9. Human skin biopsies . . . . .	62
3.10. Morphometry, statistical analyzes and data representation . . . . .	63
<b>4. Projects</b>	<b>65</b>
4.1. Role and mechanism of action of (ca)Nrf2 in UV protection . . . . .	67
4.1.1. My own contributions to this manuscript were: . . . . .	67
4.2. A novel role of Nrf3 in the UVB response of keratinocytes . . . . .	83
4.2.1. My own contributions to this manuscript were: . . . . .	83
4.3. A role for miRNAs in the acute cutaneous UVB response . . . . .	111
4.3.1. Abstract . . . . .	111
4.3.2. Introduction . . . . .	111
4.3.3. Methods . . . . .	113
4.3.4. Results . . . . .	118
4.3.5. Discussion and Outlook . . . . .	127
4.3.6. Supplemental information . . . . .	133
<b>References</b>	<b>137</b>
<b>A. Appendix</b>	<b>147</b>

Abbreviations . . . . .	149
Curriculum Vitae . . . . .	153
Publications . . . . .	155
Acknowledgments . . . . .	157