Roles of the KEAP1-NRF2 system in mammalian skin exposed to UV radiation

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ABSTRACT

The KEAP1 (Kelch-like ECH-associated protein 1)-NRF2 (NF-E2-related factor 2) system controls the biochemical defense activity against agents toxic to mammals and responds to exogenous and endogenous stressors such as electrophilic and oxidative substances, which can have destructive and genotoxic effects on affected mammalian tissues. Although this system can be activated by various environmental stressors, it remains unclear whether ultraviolet radiation (UVR), which is one of the major environmental agents that has inflammatory and carcinogenic impacts on human skin and eyes, induces NRF2-dependent defense activity. Here, we review the recent progress in the study of the contributions of NRF2 and related factors to protection against UVR. The KEAP1-NRF2 system is not always efficient in responding to UVR, especially to short wavelengths such as UVC/UVB, indicating that UVR is a poor activator of the KEAP1-NRF2 system. However, sustained activation of NRF2 appears to suppress the harmful effects of chronic UVR exposure, such as photoaging of and carcinogenesis in the skin, indicating that NRF2 activation is beneficial for the protection of the skin from the harmful effects of UVR. However, it should be noted that prolonged and strong activation of NRF2 may also have adverse effects on skin, especially in the case of UVR-induced carcinogenesis. We present working models describing mechanisms underlying the involvement of the KEAP1-NRF2 system in skin photoaging and carcinogenesis.

Keywords: NRF2, UV radiation, UVA, skin photoaging, skin carcinogenesis, antioxidation

1. Introduction

Ultraviolet radiation (UVR) is one of the most genotoxic agents that is ubiquitous in the environment. UVR can affect human skin, causing an inflammatory response of sunburn (erythema and edema) in the short term (Parrish et al., 1982) and photoaging, such as wrinkling and pigmentation, in the long term (Rittié and Fisher, 2002; Rabe et al., 2006). In the worst case scenario, UVR exposure can result in carcinogenesis (Brash, 1997). The genotoxicity of UVR originates from its capacity to produce DNA damage, which can induce mutations in the skin genome, leading to inactivation of genes necessary for skin homeostasis and activation of genes associated with oncogenic transformation (Brash, 1997; Ikehata and Ono, 2011). DNA damage can also cause apoptosis in skin tissue, leading to inflammation (Ziegler et al., 1994). UVR produces DNA damage via two distinct mechanisms. The first mechanism is direct photoreaction of UVR photons with DNA pyrimidine bases, leading to formation of dipyrimidine base adducts such as cyclobutane pyrimidine dimers (CPDs). The second mechanism is an indirect process that is mediated by production of reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), via the photosensitization of small organic molecules such as riboflavin and porphyrin (Cadet et al., 2000, 2012, 2015). ROS can induce oxidative DNA damage, such as the formation of 8-hydroxyguanine (8OH-G) (Grollman and Moriya, 1993; Cadet et al., 2000, 2015). These two types of UVR-induced DNA damage induce different types of mutation: CPD and other photolesions specifically induce C to T base substitutions at dipyrimidine sites whereas ROS-induced 8OH-G induces G to T mutations most frequently (Ikehata and Ono, 2011). Biological systems, including mammalian skin, respond to these UVR insults with protective mechanisms, such as DNA repair and damage avoidance (Marteijn et al., 2014; Friedberg et al., 2002; Livneh et al., 2016).

However, the mechanisms associated with the tolerance of UVR-mediated ROS hazards remain controversial and have yet to be elucidated (Cadet et al., 2009; Rizzo et al., 2011; Sage et al., 2012; Premi and Brash, 2016; Ikehata, 2018). One of most likely candidate mechanisms is the KEAP1 (Kelch-like ECH-associated protein 1)-NRF2 (NF-E2-related factor 2) system, which controls a battery of cytoprotective genes that encode enzymes that protect cells and tissues from oxidative and electrophilic stress of both exogenous and endogenous origins (Itoh et al., 1997; Suzuki et al., 2013; Hayes and Dinkova-Kostova, 2014; Yamamoto et al., 2018). In this review, we survey the literature regarding the contributions of the KEAP1-NRF2 system to the defense against UVR toxicity and discuss the roles of this system in the protection of mammalian skin from the harmful effects of UVR, such as photoaging and carcinogenesis.

2. Protection of the skin from UVR by the KEAP1-NRF2 system

To the best of our knowledge, the first study of the contribution of the KEAP1-NRF2 system to skin protection against UVR genotoxicity was conducted by Talalay's group using a UVR-induced skin carcinogenesis model in hairless mice (Dinkova-Kostova et al., 2006). The study demonstrated that topical and repetitive application of sulforaphane after repetitive UVR exposure activates the NRF2-controlled antioxidant response in mouse skin and suppresses the development of skin cancer, reducing tumor incidence and multiplicity as well as the total tumor volume per mouse. They also found that postirradiation dietary application of broccoli sprout extracts, which contain glucoraphanin, a precursor of sulforaphane, delays tumor development in UVR-exposed mice (Dinkova-Kostova et al., 2010). They further showed that activation of NRF2-dependent cytoprotection by sulforaphane treatment prior to UVR exposure mitigates the

inflammatory responses to UVR, such as edema and erythema, in both mouse and human skin (Talalay et al., 2007). Thus, both pre- and post-irradiation activation of NRF2 protect the skin from UVR toxicity.

These observations were partially confirmed in a study with Nrf2-knockout (Nrf2-ko) mice by Otsuka and colleagues (Kawachi et al., 2008), who reported that Nrf2-ko mice exhibit greater sensitivity to erythema and dermal edema induced by acute exposure to UVB than wild-type mice. Inflammatory cell infiltration, appearance of sunburn cells (apoptotic keratinocytes) and 8OH-G formation were also enhanced in the exposed skin of Nrf2-ko mice compared with wild-type mice. However, in contrast to the study by Talalay's group, little difference was observed in the induction of skin cancer after chronic exposure to UVB between Nrf2-ko and wild-type mice, although such chronic exposure accelerated skin photoaging in Nrf2-ko mice, inducing coarse wrinkle formation, loss of flexibility, epidermal thickening and subepidermal deposition of extracellular matrix (Hirota et al., 2011).

Subsequently, Dinkova-Kostova and colleagues confirmed the observation in the latter report, showing that no difference exists in UVR-induced skin carcinogenicity between Nrf2-ko and wild-type mice (Knatko et al., 2015). However, in the same study, they found that genetic activation of NRF2, which was induced by Keap1 deficiency, protects mice from skin carcinogenesis induced by chronic UVR exposure. Furthermore, continuous pharmacological NRF2 activation with the potent NRF2 activator TBE-31 before and during chronic UVR treatment was found to suppress the development of skin cancers as measured by tumor multiplicity and volume per mouse,. In contrast to the study by Otsuka's group, Knatko et al. (2015) showed that genetic inactivation of NRF2 did not

enhance the acute skin response to UVR, such as erythema, while the response was suppressed significantly by genetic NRF2 activation (Table 1).

The discrepancies between these findings may be attributable to the use of different UVR sources because the severity and quality of the effects of UVR depend on the wavelength (Ikehata et al., 2013). UVR is subdivided by wavelength into UVC (< 280 nm), UVB (280–320 nm) and UVA (320–400 nm), and the UVR toxicity is usually higher at shorter wavelengths although the UVC components are largely blocked by the epidermal cornified layer. The emission spectrum of the UVB lamps used by Otsuka's group was shifted to a shorter wavelength range than that of the UVR source used by Dinkova-Kostova's group, so the results in the former study should reflect the effects of shorter wavelengths, which are the more erythematic and inflammatory components of UVR.

Importantly, taken together, the results of both studies demonstrate that NRF2 plays a protective role against chronic UVR exposure. Otsuka's group showed that NRF2 is necessary for protection of the skin from photoaging, while Talalay and colleagues showed that continuous NRF2 activation suppresses or delays the development of skin cancer. Because UVB does not efficiently activate NRF2 in the skin or skin cells (Hirota et al., 2005; Durchdewald et al., 2007), it may not be surprising that no difference in skin carcinogenesis was observed between Nrf2-ko and wild-type mice after chronic UVB or solar-simulated UVR exposure. These studies indicate that, in the presence of chronic UVR exposure, continuous activation of NRF2 in normal skin is necessary to prevent or delay skin tumor growth whereas basal NRF2 activity is sufficient to suppress skin photoaging (Table 1).

3. Effects of genetic NRF2 activation on the skin

While the activation of NRF2 provides protection against UVR genotoxicity in skin tissues, continuous NRF2 activation may not always be beneficial for skin health. Werner and colleagues constructed an N-terminal-whole-Neh2-domain-deleted Nrf2 cDNA, which produces a constitutively active NRF2 (caNRF2) protein because of the lack of the ability to interact with KEAP1. They generated a series of transgenic mouse lines expressing the caNrf2 gene with a floxed stop cassette in the epidermis.

The first caNrf2 transgenic mouse line harbored the transgene under the control of the β-actin promoter and a Cre transgene that is specifically expressed in the epidermis. The transgenic mice exhibited significant reduction of apoptosis in the epidermis after UVB exposure compared to the nontransgenic control mice (Schäfer et al., 2010). With this and related transgenic mouse lines, they identified a descending gradient of NRF2 activation levels from suprabasal to basal cell layers in the epidermis. This result is consistent with the observation that suprabasal cells are more resistant to UVR-induced apoptosis than basal cells (Schäfer et al., 2010). Thus, the activation of NRF2 seems favorable for protection of the skin from UVR. Importantly, this line of transgenic mice exhibits mild hyperkeratosis in the skin.

Werner and colleagues constructed another transgenic mouse line overexpressing the caNrf2 gene under the control of a CMV enhancer/β-actin promoter specifically in the epidermis (Schäfer et al., 2012). Mice expressing caNRF2 from this transgene exhibited reduced body size and hair malformation and loss. These mice exhibited prominent anomalies in their epidermal structure, for example, epidermal thickening, hyperkeratosis, corneocyte fragility and impaired desquamation, showing a lamellar ichthyosis-like phenotype at the early ages of 1-3 months. The mice were also defective in epidermal barrier function, resulting in skin dryness and an inflammatory response, with increased

infiltration of T cells, mast cells and neutrophils into the dermis. In addition, they exhibited abnormalities of hair follicles and sebaceous glands (Schäfer et al., 2014) (Fig. 1).

Notably, the caNRF2-overexpressing mice exhibited thickening and hyperkeratosis not only in the interfollicular epidermis but also in hair follicles, developing dilatation of the infundibula, which may have been responsible for the hair malformation and loss. Hair follicle dilatation was accompanied by enlarged sebaceous glands, which further developed seborrhea and cysts upon aging, exhibiting phenotypes reminiscent of chloracne/metabolizing acquired dioxin-induced skin hamartomas (MADISH). Werner's group noted that NRF2-dependent, prolonged upregulation of three epidermis-specific genes, namely Sprr2d (small proline-rich protein 2d), Slpi (secretory leukocyte peptidase inhibitor) and Epgn (epigen), causes these abnormal skin phenotypes in transgenic caNRF2-overexpressing mice. However, the adverse effects on the skin observed in the caNRF2 mice, especially the overexpression mice, should be noted with caution because such extreme conditions of NRF2 activation would hardly be achievable in normal mice even after treatments with strong NRF2 inducers.

4. Responses of the NRF2 system to UVR in skin cells and tissues

The level of basal expression of the Nrf2 gene is relatively high in human skin (Fagerberg et al., 2014) but differs among the primary human skin cells of keratinocytes, fibroblasts and melanocytes. Basal Nrf2 gene expression is approximately twofold higher in keratinocytes than in fibroblasts and melanocytes (Reemann et al., 2014). Although NRF2 is activated post-translationally through KEAP1 inactivation by various environmental stressors (Suzuki et al., 2013; Hayes and Dinkova-Kostova, 2014), UVR

appears to be a poor activator of NRF2 (Hirota et al., 2005; Durchdewald et al., 2007; Marrot et al., 2008; Kokot et al., 2009). The available lines of evidence indicate that UVR, especially UVB, downregulates the activity of NRF2 and expression of the corresponding target genes in skin fibroblasts and keratinocytes in vitro and in skin tissues in vivo (Table 2). Because UVR produces DNA base damage such as CPDs that strongly inhibit transcription (Kwei, et al., 2004; Scicchitano et al., 2004; Tornaletti, 2005), UVR would consequently retard the activation of NRF2-mediated transcription. Whereas UVR can also produce ROS, such DNA damage occurs instantly upon irradiation via direct photochemical mechanisms prior to ROS generation and acts to prevent the prompt and efficient upregulation of NRF2-target genes (Fig. 2). Although the possibility of NRF2-specific transcriptional regulation against UVR cannot be excluded, the remarkable reduction of nuclear NRF2 levels after UVB exposure (Hirota et al., 2005) suggests that the KEAP1-dependent degradation depletes NRF2 because of the cessation of NRF2 synthesis by the UVR-mediated general transcriptional block. In fact, downregulation of Nrf2 gene expression by UVB was observed in primary human keratinocytes by RNA-seq analysis (Shen et al., 2017). Interestingly, Williamson et al. (2017) proposed a short transcript-dependent mechanism of recovery from the general transcriptional block by UVR, which would aid the reactivation of the NRF2 pathway.

Notably, however, there are a few reports indicating that UVA induces NRF2-dependent cytoprotection because UVA produces transcription-blocking DNA damage much less efficiently than UVB (Setlow, 1974; Matsunaga et al., 1991) (Fig. 2). In this regard, Tyrrell and colleagues reported that a 32-kDa protein, which has now been identified as heme oxygenase 1 (HO-1), is induced in human skin fibroblasts after exposure to UVA (Keyse and Tyrrell, 1987, 1989). HO-1 is a stress-inducible

antioxidative enzyme that is transcriptionally regulated under the control of NRF2 (Ishii et al., 2000; Alam et al., 1999), whereas hypoxia or ROS-mediated, NF-κB or HIF-1-dependent pathways are also known to upregulate HO-1 (Wu et al., 2004; Ryter and Choi, 2005; Lien et al., 2014). Tyrrell's group confirmed that UVA-mediated HO-1 induction is NRF2 dependent and protects cells from membrane damage caused by UVA (Zhong et al., 2010). HO-1 induction by UVA is not confined to fibroblasts, as this phenomenon has also been detected in human keratinocytes by another group (Marrot et al., 2008). In addition, Tyrrell's group also demonstrated that glutathione (GSH) depletion sensitizes cultured human keratinocytes and fibroblasts to UVR, especially to longer wavelengths in the UVA range, and that this sensitization is more prominent for fibroblasts than for keratinocytes (Tyrrell and Pidoux, 1988). This study could be another example of the contribution of the KEAP1-NRF2 system to the protection against UVR toxicity because the cellular GSH level is known to be controlled by GSH-metabolizing enzymes, such as glutamate-cysteine ligase (GCL), cystine/glutamate transporter xCT and GSH S-transferases, many of which are regulated by NRF2 (Suzuki et al., 2013; Hayes and Dinkova-Kostova, 2014). The relatively poor sensitization of keratinocytes to UVR by the less efficient GSH depletion has been reproduced in mice lacking the modifier subunit of GCL, in which the GSH level is reduced by 70% in the skin, as shown by the frequencies of UVR-induced sunburn cells (Telorack et al., 2016a).

Direct evidence for the UVA-mediated activation of NRF2 was first reported by Otsuka and colleagues (Hirota et al., 2005). They detected significant NRF2 accumulation in the nuclei of mouse dermal fibroblasts in vitro after exposure to UVA but not UVB (Table 2). NRF2 upregulation suppresses UVA-induced apoptosis of dermal fibroblasts, which they demonstrated by using Nrf2-ko and Keap1-ko mutant cells (Table

1). Gruber et al. (2010) also demonstrated NRF2 upregulation by UVA in human dermal fibroblasts, which is mediated by UVA-oxidized phospholipids. In addition, NRF2 accumulation was observed in melanocytes and melanoma cells after UVA exposure, and the accumulated NRF2 further upregulated the autophagy receptor adaptor p62 in an ROS-production-dependent manner (Sample et al., 2018). On the other hand, Werner's group reported that UVA, as well as UVB, does not activate the KEAP1-NRF2 system in mouse keratinocytes in vitro and in vivo (Durchdewald et al., 2007) (Table 2), although this observation does not necessarily exclude a protective ability of NRF2 against UVR. In fact, NRF2 activation prior to irradiation with electrophilic chemicals protects keratinocytes from UVA-induced apoptosis (Kimura et al., 2009) (Table 1). The differences in the NRF2 responses to UVA between Werner's study and the others might be due to differences in the cell types used or due to the use or lack thereof of filters that cut off the shorter wavelengths in the UVB range emitted from UVA sources. Because the photochemical and photobiological effectiveness of UVB is much more powerful than that of UVA, UVB contamination, which would be expected in the study by Werner's group, could mask the effects of UVA (Ikehata et al., 2013) (Fig. 2).

Thus, UVA seems to be able to activate the KEAP1-NRF2 system, at least in skin fibroblasts in vitro (Hirota et al., 2005; Zhong et al., 2010; Gruber et al., 2010) and in melanocytes (Marrot et al., 2008; Sample et al., 2018). However, the NRF2 upregulation observed in these studies was often dependent on the presence of heme; the repletion and depletion of heme in cells strongly stimulates and suppresses the upregulation, respectively (Hirota et al., 2005; Zhong et al., 2010). Porphyrin is known to be a potent photosensitizer that is activatable by visible light and UVA and can produce $^1O_2$ (Cadet et al., 2000). It has also been reported that hydrogen peroxide (H_2O_2) and oxidized

phospholipids can activate HO-1 and the KEAP1-NRF2 system in fibroblasts (Keyse and Tyrrell, 1989; Gruber et al., 2010; Saito et al., 2016). Hence, NRF2 activation by UVA most likely occurs due to ROS production via UVA-activatable photosensitizing molecules such as porphyrin and riboflavin (Fig. 2). Because the levels of photosensitizable ingredients in cultured media influence the cellular levels of ROS after UVA exposure (Besaratinia et al., 2007), cell culture conditions could artificially affect the extent of NRF2 activation by UVA.

In contrast to the results observed with fibroblasts, NRF2 activation is not always observed in keratinocytes after UVA exposure or even after H_2O_2 treatment, although these treatments strongly increase the intracellular ROS level (Durchdewald et al., 2007) (Table 2). The reason why keratinocytes and fibroblasts respond differentially to UVA is currently unknown. One possibility is that keratinocytes possess a more secure antioxidant ability than fibroblasts. In fact, the level of GSH and the resistance to the GSH synthesis inhibitor buthionine-S,R-sulfoximine (BSO) were reported to be higher for human keratinocytes than for dermal fibroblasts (Tyrrell and Pidoux, 1988). It was also reported that UVA reduces cellular GSH levels more drastically in human fibroblasts than in keratinocytes (Niggli and Applegate, 1997). Interestingly, conditional Gclc-ko mice that lack the catalytic subunit of GCL and are completely deficient in GSH synthesis in the epidermis exhibit an increased level of keratinocyte apoptosis even in the absence of UVR exposure (Telorack et al., 2016b), indicating an essential role of GSH in the maintenance of healthy skin.

Regarding the observations that UVB does not activate, or often downregulates, the KEAP1-NRF2 system in keratinocytes in vitro and in vivo (Durchdewald et al., 2007; Kokot et al., 2009) and even in fibroblasts in vitro (Hirota et al., 2005) (Table 2), Kokot

et al. (2009) made the intriguing finding that α-melanocyte-stimulating hormone (α-MSH), which is induced by UVR in a p53-dependent manner (Cui et al., 2007), can activate the KEAP1-NRF2 system by itself to counteract NRF2 downregulation by UVB in keratinocytes in vitro and in skin ex vivo organ culture. α-MSH was also found to protect skin cells from UVR-induced apoptosis by upregulating DNA repair for CPD removal (Böhm et al., 2005; Kadekaro et al., 2005; Dong et al., 2010). Because keratinocytes can secrete α-MSH and can impact their own behavior via autocrine and paracrine mechanisms, it is conceivable that UVR-induced α-MSH can protect cell populations such as the skin epidermis in which cells adhere closely to each other, by infiltrating the whole tissue and activating DNA repair and the KEAP1-NRF2 system. The α-MSH-mediated protection would be much more efficient for such a closely congregated cell population than for a scattered or less closely distributed population, such as cultured cells or the fibroblasts in the dermis. Further studies are expected to clarify these points.

5. The role of NRF2 in the protection of skin against photoaging

NRF2 can prevent skin photoaging, which is a harmful effect of UVR that affects the appearance of individuals as well as their skin health (Hirota et al., 2011). UVR is known to promote skin photoaging via upregulation of matrix metalloproteinases (MMPs) that degrade the extracellular matrix in the skin dermis by cleaving collagen and elastin, resulting in destruction of the dermal matrix that can cause deep wrinkles in the affected skin (Herrmann et al., 1993; Fisher et al., 1996; Brenneisen et al., 1996). In addition, it has been demonstrated that MMPs are also induced by $^1O_2$ (Wlaschek et al., 1995), which can be produced in cells and tissues after UVA exposure (Cadet et al., 2000).

As shown in Fig. 3, MMP upregulation is mediated by the activation of growth-factor and cytokine receptors that possess intracellular tyrosine kinase domains, which are negatively regulated by protein tyrosine phosphatases (PTPs). The activated tyrosine kinases of the receptors upregulate downstream signaling cascades such as the MAP kinase cascade, which then mediates the induction of MMP gene expression by activating the transcription factor AP-1 (Fisher et al., 1996). UVR induces MMP activity in the dermis by inactivating the PTPs via the oxidation of a critical active-site cysteine residue (Denu and Tanner, 1998; Rittié and Fisher, 2002). PTP inactivation by ROS is suppressed by peroxiredoxin (PRX) (Choi et al., 2005), which is recycled in the redox state by thioredoxin (TRX), TRX reductase 1, and sulfiredoxin (SRX) (Neumann et al., 2009) (Fig. 3, right). Recently, it was also reported that TRX reductase 1 itself can directly protect PTPs (Dagnell et al., 2017). Because some of the genes encoding PRX (PRDX1 and PRDX6), TRX, TRX reductase and SRX have been shown to be targets of NRF2 (MacLeod et al., 2009; Malhotra et al., 2010; Agyeman et al., 2012; Chorley et al., 2012; Hirotsu et al., 2012; Jung et al., 2013; Hawkes et al., 2014), the KEAP1-NRF2 system is expected to prevent skin photoaging by suppressing MMP induction via the protection of PTPs from the oxidative inactivation mediated by UVR.

Interestingly, Scharffetter-Kochanek's group has shown that the UVR-induced activation of MMPs in human dermal fibroblasts is mediated through autocrine action by interleukin-6 (IL-6), whose production is upregulated by NF-κB signaling. They observed that NF-κB/IL-6 signaling is activated through UVR-mediated intracellular or extracellular ROS production (Wlaschek et al., 1993, 1997; Brenneisen et al., 1999; Wenk et al., 2004). In these studies, they indicated that UVA-generated extracellular $^1O_2$ induces lipid peroxidation in the cell membrane and that lipid peroxidation produces

phosphatidylcholine hydroperoxide, which upregulates the NF-κB/IL-6 signaling pathway in human dermal fibroblasts and eventually leads to MMP induction. Importantly, UVA-mediated MMP induction is abrogated by the overexpression of phospholipid-hydroperoxide glutathione peroxidase or glutathione peroxidase 4 (GPX4) (Wenk et al., 2004). GPX4 might be activatable by the KEAP1-NRF2 system because a NRF2-MAFG binding site has been detected in the vicinity of the mouse Gpx4 gene (Hirotsu et al., 2012). Thus, NRF2 might be able to suppress skin photoaging via another NRF2-dependent antioxidative pathway involving GPX4 (shown to the left in Fig. 3) in addition to the pathway involving TRX and PRX (shown to the right in Fig. 3).

6. Involvement of NRF2 in the prevention and promotion of skin carcinogenesis

The mutagenicity and apoptosis inducibility of UVR are both thought to contribute to its carcinogenicity: the former produces oncogenic mutations necessary for tumorigenic initiation, whereas the latter causes tumor-promoting inflammation (Ziegler et al., 1994). As mentioned above, UVR induces these mutations in two different ways, namely, via ROS-dependent and independent pathways, and the former would be prevented by NRF2 activation (Fig. 2). Similarly, UVR causes apoptosis via two different mechanisms, i.e., ROS-dependent and ROS-independent mechanisms. The start-up kinetics of these mechanisms differ, with the former occurring much earlier than the latter (<0.5 h vs. >4 h) (Godar, 1999). The latter apoptotic process (delayed apoptosis) is induced by the formation of DNA damage such as CPD, whereas the former (immediate apoptosis) is triggered by ROS such as $^1O_2$, which are produced by UVR-mediated photosensitization and can damage the mitochondrial membrane (Godar, 2000; Simon et al., 2000). These processes also show a wavelength dependence; delayed apoptosis is caused mainly by

short wavelengths, such as UVC and UVB, whereas immediate apoptosis is induced by UVA, especially the longer range UVA1 (340–400 nm) (Godar and Lucas, 1995). The antioxidative activities induced by NRF2 would be expected to suppress the ROS-mediated immediate apoptosis triggered by UVA exposure. Thus, NRF2 activation can suppress the development of UVR-induced skin cancers by preventing ROS-mediated oncogenic mutations and apoptosis in the skin (Fig. 2). Although apoptosis can eliminate damaged cells that could transform into precancerous cells if they survived, apoptosis also produces empty spaces in affected tissues, which would allow the surrounding, less damaged cells to proliferate to fill the spaces. Such proliferation of lightly damaged cells could induce mutations including oncogenic ones. Mutation induction by hyperplasia in mouse skin epidermis after UVR-induced apoptosis has been reported (Ikehata et al., 2010).

In fact, sustained NRF2 activation protects the skin from UVR-induced carcinogenesis, as mentioned above (Dinkova-Kostova et al., 2006, 2010; Knatko et al., 2015). However, the underlying mechanism remains under investigation. The roles of the KEAP1-NRF2 system in skin carcinogenesis were explored by Schäfer and colleagues using chemical and genetic models of skin carcinogenesis (Rolfs et al., 2015), in which the low epidermal expression caNrf2-transgenic mice mentioned above were utilized (Schäfer et al., 2010). They found that NRF2 contributes to skin cancer development differently in these different models. In the chemical model of carcinogenesis, in which mice were treated once with the mutagen DMBA (7,12-dimethylbenz[*a*]anthracene) and repeatedly thereafter with the tumor promoter TPA (12-*O*-tetradecanoylphorbol 13-acetate), NRF2 activation suppressed tumorigenesis, although the extent of suppression was low. By contrast, NRF2 activation remarkably promoted tumorigenesis in the genetic model, in

which mice harbored transgenic HPV8 (human papilloma virus 8) oncogenes expressed specifically in the epidermis. The difference between the chemical and genetic models was the frequency of precancerous cells in the epidermis at the beginning of the experiment; the frequency was low or nearly zero in the former but 100% in the latter, with active oncogenes present in all cells. Because they also demonstrated that NRF2 activation does not promote cell proliferation but does promote cell survival by suppressing ROS-induced apoptosis, they argued that NRF2 activation should favor the development of skin cancer by protecting precancerous keratinocytes from ROS-induced apoptosis. The protective capacity of NRF2 activation toward cancer cells has also been reported in previous studies (Wang et al., 2008; Jaramillo and Zhang, 2013).

However, the contribution of noncancerous, normal populations of keratinocytes should not be ignored in the chemical model of carcinogenesis because the KEAP1-NRF2 system is also upregulated as highly in the normal population as in the small number of initiated precancerous keratinocytes. The lower efficiency of tumorigenesis in the NRF2-activated mice than in the control mice in the chemical model of carcinogenesis could be the result of a competition between normal and precancerous keratinocytes in the epidermis, which suggests that NRF2 activation can confer an advantage to normal cells to counteract precancerous cells (Fig. 4, left). The study by Knatko et al. (2015) supports this idea because mice with genetic activation of NRF2 exhibited greater resistance to UVR-induced skin carcinogenesis than wild-type mice, which indicates that NRF2-activated normal keratinocytes can also compete with UVR-initiated precancerous cells more effectively than nonactivated keratinocytes. The advantage of sustained NRF2 activation for the prevention of carcinogenic initiation has also been demonstrated in a mouse model of urethane-induced lung cancer using Keap1-knockdown (kd) mice, which

express a high level of NRF2 (Satoh et al., 2016). Thus, continuous NRF2 activation during carcinogenic treatments in normal cells, whether it is achieved genetically (e.g., caNrf2 or Keap1-kd) or pharmacologically (e.g., sulforaphane or TBE-31), is expected to suppress the expansion of precancerous cells in the tissue.

Importantly, the lung cancer study by Satoh et al. further provided evidence that NRF2 activation accelerates tumor development to the contrary once the cells have transformed into a tumorigenic state, by testing in nude mice the tumorigenicity of the urethane-induced lung tumor cells derived from the Keap1-kd mice (Satoh et al., 2016), a condition that is similar to the carcinogenically initiated keratinocytes in the genetic model of skin cancer mentioned above (Rolf et al., 2015). UVR causes not only mutations, some of which could be oncogenic, but also apoptotic cell death in the affected skin tissue, which can induce hyperplastic cell proliferation to reconstitute the damaged tissue (Ikehata et al., 2010). Repetitive UVR exposure, which is required to induce skin cancer, would cause inflammation, a condition in which simultaneous cell division and death occur perpetually, through inflammasome activation (Feldmeyer et al., 2007; Nasti and Timares, 2012). In such inflammatory conditions, there is competition among surviving cells, some of which acquire oncogenic mutations during proliferation, finally leading to the formation of skin cancer, which is almost an inevitable outcome as long as the inflammation does not subside (de Gruijl and Forbes, 1995). Prolonged inflammation favors cancer development because such sustained inflammatory states accumulate oncogenic mutations, which could synergistically enhance the proliferation and survival of the affected cells, i.e., initiated cells (Ziegler et al., 1994; Jonason et al., 1996) (Fig. 4, right). Immediate and efficient damping of inflammation is therefore necessary to

suppress skin cancer development by UVR. NRF2 activation is known to inhibit the inflammasome and consequently inflammation (Hennig et al., 2018).

As mentioned previously, Schäfer and colleagues proved that the promotion of skin tumorigenesis by NRF2 activation occurs due to the improvement of the survival of cancer cells by the suppression of ROS-induced apoptosis, not due to the enhancement of proliferation (Rolf et al., 2015). They further provided evidence that the improvement of survival would be brought about by a metabolic adaptation to the inflammatory/tumorigenic microenvironment that can expose cells to apoptotic stimuli such as ROS (Rolf et al., 2015). It is known that NRF2 activation causes a metabolic alteration in affected cells, upregulating the pentose phosphate pathway and GSH synthesis, which increases the intracellular ATP/GTP, NADPH and GSH levels, thereby enhancing cell proliferation and survival by providing energy sources and antioxidative conditions (Mitsuishi et al., 2012).

This metabolic reprogramming can also be beneficial for uninitiated, normal cells, at least at the early stages of carcinogenesis, because these cells overwhelmingly outnumber the initiated precancerous cells, so that they can rapidly occupy empty spaces in the tissue resulting from apoptosis and prevent the expansion and further mutagenic transformation of precancerous cells, as evidenced by the observations for NRF2-activated mice in the models of urethane-induced lung tumorigenesis (Satoh et al., 2016) and UVR-induced skin carcinogenesis (Knatko et al., 2015). In contrast, at the later stages, NRF2 activation would be rather beneficial for precancerous/cancerous cells because oncogenic mutations provide these cells with increased proliferation and survival abilities that can be enhanced by NRF2 upregulation (Fig. 4). Nrf2-activating mutations themselves have been detected

as candidate oncogenic mutations in various human cancers (Yamamoto et al., 2018; De la Vega et al., 2018).

Although it has also been reported that functional NRF2 is necessary to support the stable growth of lung tumors without regression (Satoh et al., 2013), this requirement for NRF2 function was not observed for UVR-induced skin cancers (Kawachi et al., 2008; Knatko et al., 2015). This discrepancy might originate from the different modes of carcinogen treatment: urethane was administered by a single dose only at the beginning of the experiment, whereas UVR was provided repetitively for a fairly long period in the earlier steps of the experiment, which suggests some activity of UVR for tumor promotion that is equivalent to the function of NRF2 necessary for the maintenance of lung tumorigenesis. In fact, it was reported that UVR-induced precancerous keratinocyte patches in the epidermis that harbored presumed p53 mutations regressed remarkably after discontinuation of the repetitive exposure (Berg et al., 1996).

7. Conclusion

UVR affects the genome and tissue of the skin due to its DNA-damaging and ROS-producing abilities, which cause mutations and apoptosis in the skin, leading to photoaging and carcinogenesis after repetitive exposure. This toxicity of UVR toward the skin is mediated by two pathways, i.e., ROS-dependent and ROS-independent pathways. The latter is triggered by DNA damage formed by direct photoreaction with DNA. The effects of the latter pathway are induced mainly by the short wavelengths of UVR, namely, UVC and UVB, whereas those of the former pathway are brought about not only by UVC/UVB but also by the long-wavelength component UVA, which all produce ROS via photochemical activation of photosensitizable biomolecules such as porphyrins. The

KEAP1-NRF2 system can prevent or mitigate the harmful effects of UVR caused by the ROS-dependent pathway by activating a battery of antioxidative activities, although this system seems unable to prevent the ROS-independent toxicity of UVR. The ROS-dependent UVR toxicity toward the skin is involved in photoaging and cancer formation, both of which can be suppressed by the activation of NRF2. NRF2 activation could suppress the initiation step of carcinogenesis by preventing ROS-induced mutations and apoptosis and enhancing the survival of noncancerous normal cells, although prolonged activation would be rather detrimental to skin cancer prevention because it preferentially promotes the survival of precancerous cells that have acquired oncogenic mutations advantageous for proliferation in the affected skin. Precise evaluation of the role of KEAP1-NRF2 system in the control of ROS-dependent UVR toxicity for the skin still requires detailed studies on the downstream pathways of the system that function in the skin to prevent UVR-induced mutation, apoptosis, inflammation and photoaging, whereas consideration for the ROS-independent pathway is also necessary to estimate the exact contribution of NRF2 to skin protection against the overall harmfulness of UVR.

Conflict of interests

There are no conflicts of interest to declare.

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Figure captions

Fig. 1. Effects of genetic NRF2 activation on the skin. According to studies by Werner and colleagues, genetically activated NRF2 can protect skin against environmental toxic agents at low expression levels, whereas extremely high expression of this gene has various adverse effects on the skin, such as anomalies in the structure of the epidermis, hair follicles and sebaceous glands that can develop into seborrhea and cysts upon aging.

Fig. 2. Involvement of NRF2 in the ROS-dependent and ROS-independent pathways that mediate the toxicity of UVR toward the skin. The toxic effects of UVR are wavelength dependent. Short wavelengths in the UVC/UVB range produce DNA base damage via direct photoreaction with DNA (ROS-independent pathway), whereas this effect decreases substantially at wavelengths in the UVA range, which exert toxicity mainly via ROS production by photosensitizing biomolecules in cells and tissues (ROS-dependent pathway). This UVR-produced DNA damage and ROS induce mutations and apoptosis in affected tissues irrespective of the pathway and can cause inflammation, photoaging and cancers in the skin. The activation of NRF2 can protect against UVR toxicity by upregulating a battery of antioxidative activities to counteract the ROS-dependent pathway; however, NRF2 activation has little suppressive effect on the UVR toxicity due to the ROS-independent pathway because the potent ability of UVC/UVB to produce DNA base damage leads to downregulation of NRF2 via transcriptional inhibition.

Fig. 3. Schematic diagram of a hypothetical cellular mechanism for the control of skin photoaging. Repetitive UVR exposure causes skin photoaging by upregulating matrix

metalloproteinases in dermal fibroblasts. ROS-mediated intra/intercellular signaling pathways are thought to be involved in the upregulatory mechanism. A group of antioxidative enzymes, many of which are under the control of NRF2, can suppress photoaging by reducing the levels of UVR-induced ROS and lipid peroxidation products. aPTP, active protein tyrosine phosphatase; GPX, glutathione peroxidase; iPTP, inactive PTP; LPO, lipid peroxidation; MMPs, matrix metalloproteinases; PCOOH, phosphatidylcholine hydroperoxide; PRX, peroxiredoxin; PTK, protein tyrosine kinase; SRX, sulfiredoxin; TRX, thioredoxin.

Fig. 4. Hypothetical model of NRF2 involvement in UVR-induced skin cancer development. UVR induces skin cancers via ROS-dependent (black dashed arrows) and ROS-independent (gray dashed arrows) pathways, both causing mutations and apoptosis. Although apoptosis can eliminate damaged cells that could otherwise transform into precancerous cells, apoptotic tissue destruction also induces cell proliferation, which usually restores the skin tissue to its normal state while simultaneously being able to induce mutations. The mutations induced directly by UVR and indirectly during such proliferation are likely to include oncogenic mutations, which often promote the proliferative ability of affected cells (the initiation step of tumorigenesis). Once such oncogenic mutations are acquired, cells become precancerous, attaining an increased ability to proliferate and survive compared to other normal cells in the tissue (red arrow). Such precancerous cells acquire additional oncogenic mutations directly or by repeated proliferation in response to apoptosis during repetitive UVR exposure, gradually acquiring increasing proliferative abilities that are sufficient to outcompete the surrounding normal or low-potency precancerous cells (the promotion step). The KEAP1-

NRF2 system can prevent the initiation step by suppressing ROS-induced mutations and apoptosis after UVR exposure but can also enhance cancer development at the promotion step by protecting precancerous/cancerous cells from ROS-mediated apoptosis.

Table 1

Summary of studies on the protection of the skin against the toxic effects of UVR by NRF2.

UVR toxicity	Basal NRF2	Activated NRF2	
		Chemical	Genetic
Acute			
Apoptosis	+ (fibroblast)[1]	+ (keratinocyte)[2]	+ (fibroblast)[1]
Inflammation (erythema/edema)	+[3] or –[4]	+[5]	+[4]
Chronic			
Photoaging	+[6]	ND	ND
Carcinogenesis	–[3,4]	+[4,7]	+[4]

+: significant/marked protection; -: no significant/marked protection; ND: not determined. [1] Hirota et al., 2005; [2] Kimura et al., 2009; [3] Kawachi et al., 2008; [4] Knatko et al., 2015; [5] Talalay et al., 2007; [6] Hirota et al., 2011; [7] Dinkova-Kostova et al., 2006, 2010.

Table 2

NRF2 responses to UVB and UVA in different skin cell types.

	NRF2 activation in		
	Keratinocytes	Fibroblasts	Melanocytes
UVB	–[1,2]	–[3]	–[2]
UVA	–[1] or +[4]	+[3,5,6]	+[4,7]

+: significant/marked NRF2 activation; -: no significant/marked activation.

[1] Durchdewald et al., 2007; [2] Kokot et al., 2009; [3] Hirota et al., 2005; [4] Marrot et al., 2008; [5] Gruber et al., 2010; [6] Zhong et al., 2010; [7] Sample et al., 2018.

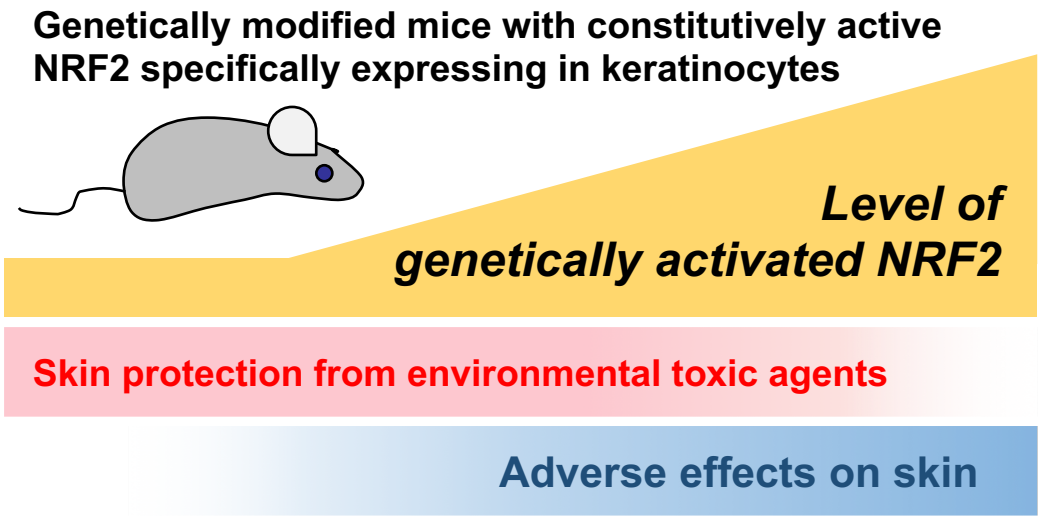

Fig. 1. Ikehata and Yamamoto

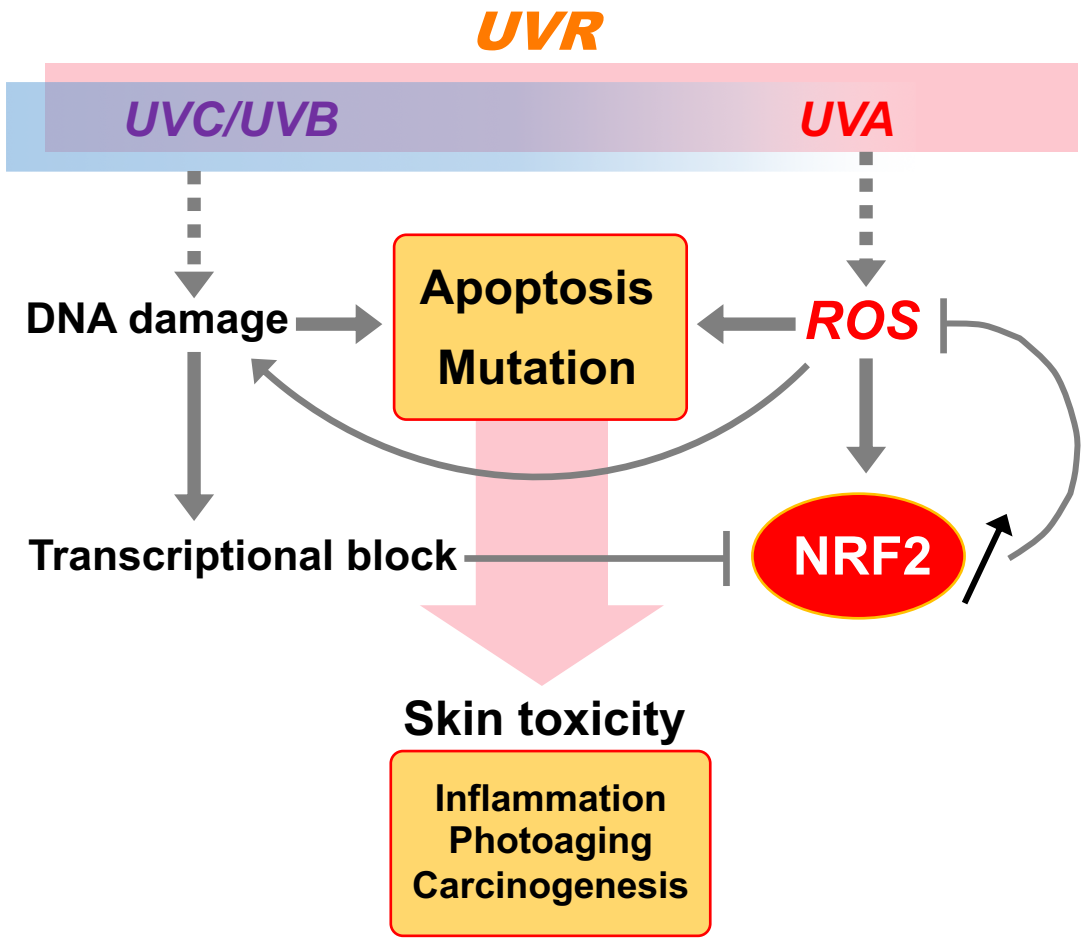

Fig. 2. Ikehata and Yamamoto

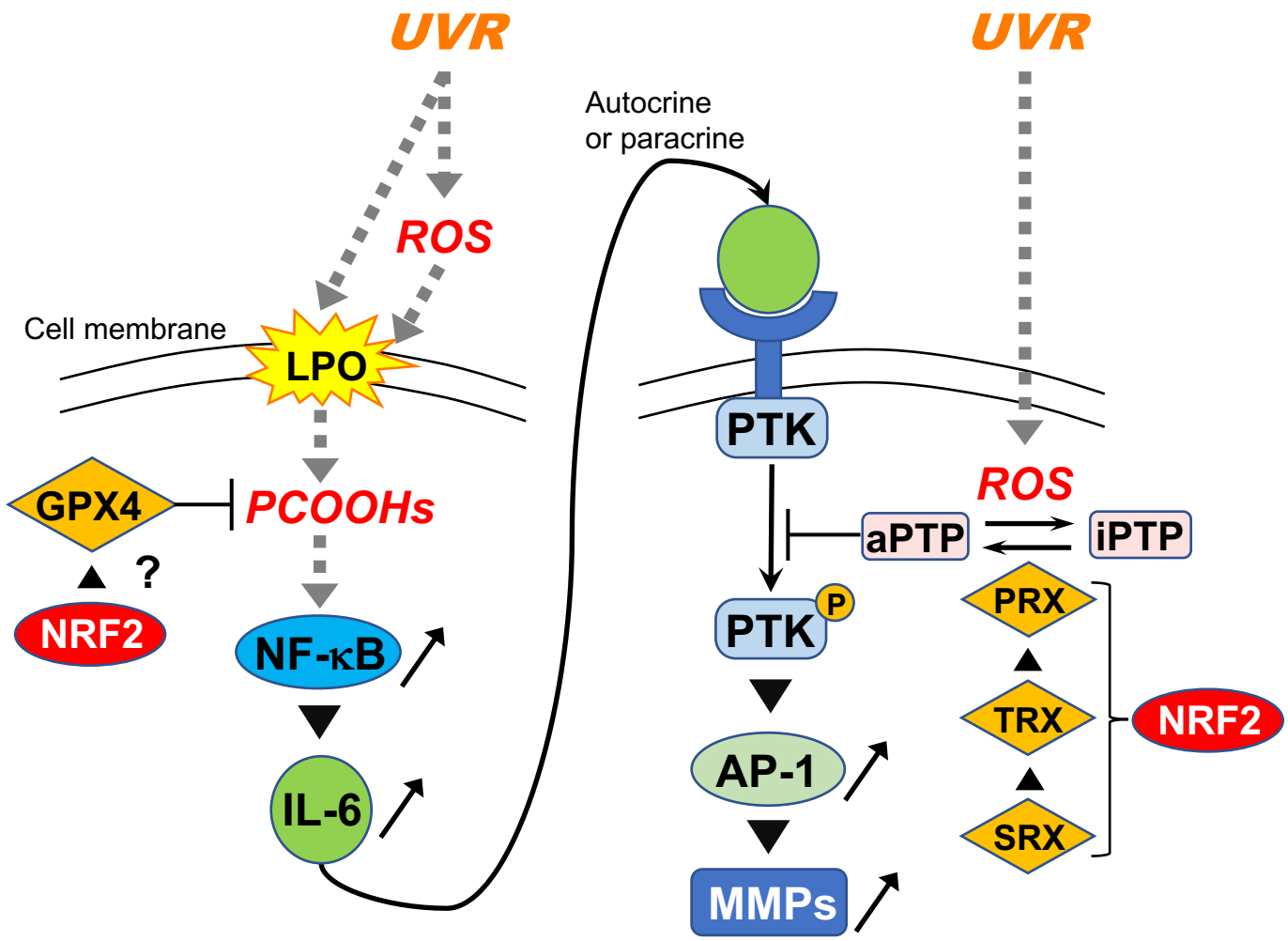

Fig. 3. Ikehata and Yamamoto

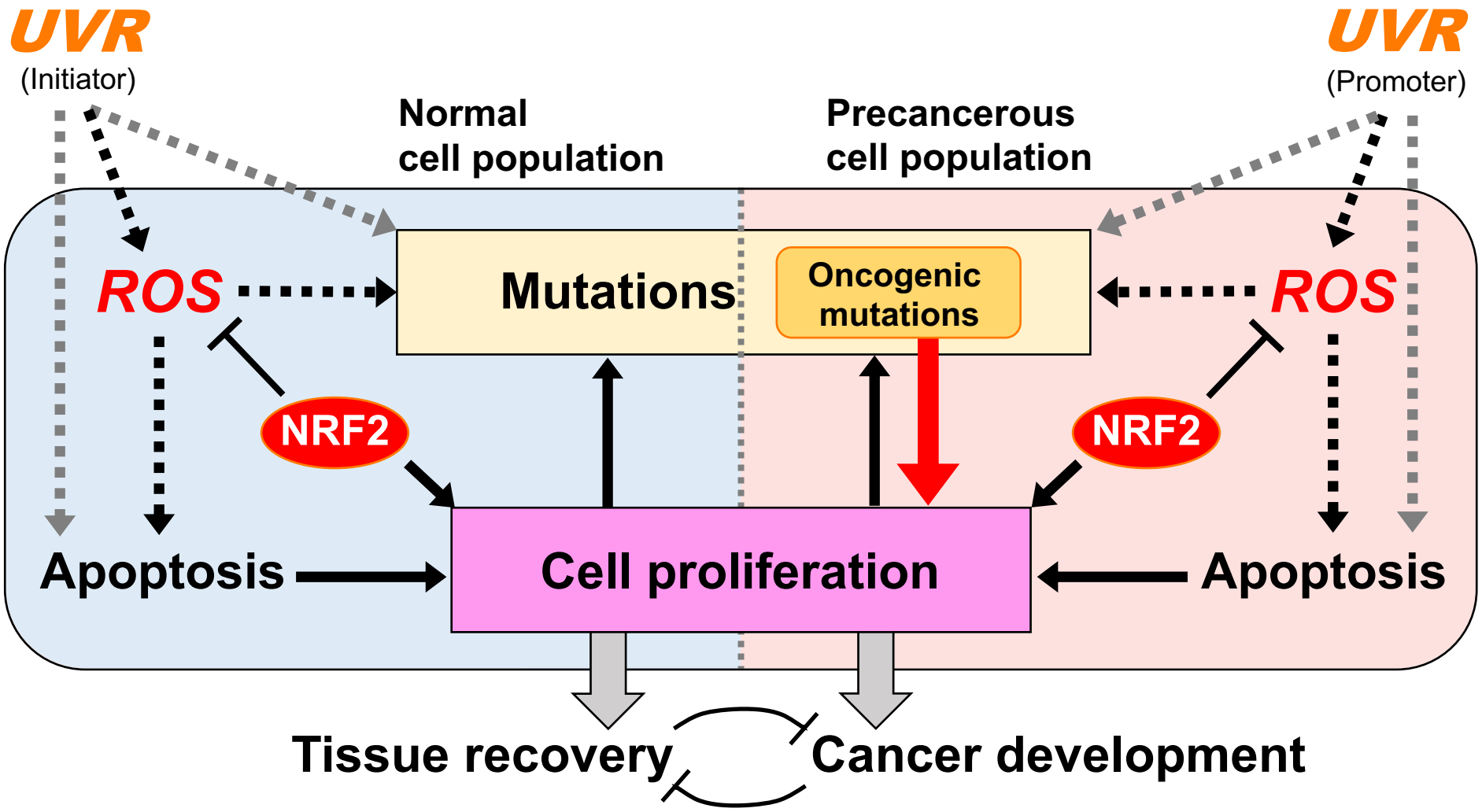

Fig. 4. Ikehata and Yamamoto