Tanning bed use, risk of melanoma and opportunity for prevention with sulforaphane

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Abstract: The use of indoor tanning beds for cosmetic purposes has been very popular among the Caucasian population in the US. It is estimated that almost one in three Caucasian women aged 16 to 25 years use indoor tanning devices each year. Indoor tanning beds produce concentrated UV-A and UV-B rays and can be more harmful than the natural rays of the sun. The International Agency for Research on Cancer classified UV-emitting tanning devices as group 1 carcinogens in 2009. UV exposure from sunlight and indoor tanning bed has been linked to the risk of melanoma and non-melanoma skin cancers. Although the incidence rate of melanoma is higher among men than women, melanoma is more common among women among those aged 50 years or younger, which can be, at least partially, attributed to the use of indoor tanning beds. Recently, it has been reported that volunteers subjected to UV light and treated with sulforaphane showed a decrease in the development of skin erythema. Sulforaphane is a natural compound that is present abundantly in broccoli and broccoli sprouts. Commercially available sulforaphane supplements have been demonstrated to be safe and lack of toxicity. Data indicates that sulforaphane has anti-cancerous activity via a reduction of glutathione levels, which is regulated by nuclear factor erythroid 2-related factor-2 (Nrf2). The knowledge gained so far may present a unique opportunity for chemoprevention trials examining the efficacy of sulforaphane in melanoma prevention. Well-planned and well-characterized trials examining molecular pathways could also further our understanding of the carcinogenic mechanisms for melanoma.

Keywords: Melanoma; tanning bed; sulforaphane; nuclear factor erythroid 2-related factor-2 (Nrf2); cancer prevention

Epidemiology of melanoma

Cancer of the skin is the most common of all cancers. Skin cancers that are not melanomas, such as basal cell and squamous cell, are often grouped as non-melanoma skin cancers because they develop from skin cells other than melanocytes and tend to behave very differently with minimal tendency for metastasis compared to melanoma. Melanoma accounts for only about 1% of skin cancers but it represent the majority of skin cancer deaths. The American Cancer Society estimated that about 76,380 new melanoma cases, 46,870 men and 29,510 women, will be diagnosed in 2016 in the United States and 10,130 deaths (6,750 men and 3,380 women) are predicted (1). Overall, rates of melanoma incidence are approximately 60% higher among men than women. However, among persons aged less than 50 years, melanoma is more common among women (2). According

to the Surveillance, Epidemiology, and End Results (SEER) Program, the annual incidence rate of melanoma among whites increased by more than 60 percent from 1991 to 2011 (3).

Risk factors for melanoma

There are many known risk factors of melanoma. High lifetime sun exposure is one of the most apparent factors, as UV-A and UV-B rays damage skin cells and induce tumors and cancer cell growth [reviewed in (4)]. People who live in southern areas of the US and are repeatedly exposed to UV-A and UV-B rays are at a higher risk. People who are fair skinned, Fitzpatrick type I–II, and burn easily are also more prone to these effects. People have a history of blistering sunburns also have higher risk for melanoma (3). If an individual has a history of melanoma, or any other carcinomas, there is an increased chance of recurrence (5). Family history is strongly linked with melanoma (6-8); approximately one in every ten individuals with melanoma report a family member with a melanoma diagnosis. Moles, more specifically, atypical moles, are common precursors to melanoma. The more moles and the greater the atypical features, the higher the risk for melanoma (9,10). Genetic factors have also been shown to contribute significantly to melanoma risk [reviewed in (4,11)]. Mutation of the *BRAF* gene is found in approximately half of all melanomas [reviewed in (12)], and is one the best-defined molecular abnormalities contributing to the pathogenesis of melanoma. Mitogen-activated protein kinase (MAPK) extracellular signaling pathway is induced by *BRAF* mutation, thus promoting the proliferation of tumor cells. Due to the recent advances in cancer genomics, germline variants in *CDKN2A* and *CDK4*, *TERT*, *MITF*, and *BAP1* have been added to the list of genes harboring melanoma pre-disposing mutations (8).

Use of tanning beds in the United States

Indoor tanning beds produce concentrated UV rays and can be more harmful than the natural rays of the sun (13-16). For the past three decades, the use of tanning beds has become very popular among Caucasian populations, particularly among younger women. An estimated 11.6 million persons in the United States, including almost one in three Caucasian women aged 16 to 25 years, use indoor tanning devices each year [reviewed in (2,17)]. Indoor tanning beds use two types of rays, UV-A and UV-B, both of which can lead to skin cancers. These tanning beds are designed for short duration of use, so the bulbs emit high intensity in the short amount of time they are in use. There has been claims by the tanning device industry that newer tanning devices employ newer electronic ballasts versus previous version of magnetic ballasts, which "virtually eliminate risk (of melanoma) and are safe" (18,19). Tanning beds use, regardless of the old or new model, are at high risk for causing melanoma and other harmful effects. Because the duration and frequency of use of tanning beds is positively correlated with risk, those who tan at younger ages are at greater risk for developing melanoma, in part because the skin is still developing. When controlling for outdoor sun exposure, there is strong evidence that younger users have an increased risk for development of melanoma (20-22). Dose-dependency also factors into each person's individual risk for melanoma development. This risk depends on the length, strength, and duration of the tanning beds used (23). UV from indoor tanning devices has been classified by the World Health Organization and the U.S. Department of Health and Human Services as a known carcinogen (24).

A meta-analysis published in the British Association of Dermatologists concluded that an overall summary relative risk (RR) of 1.20 [95% confidence interval (CI), 1.08–1.34] for melanoma development in 'ever use' of tanning beds and a 1.8% increase of risk for each additional session of sunbed use per year (25). In a subgroup analysis of subjects who first used sunbeds at an age below 35 years, the summary RR rose to 1.87 (95% CI, 1.41–2.48) indicating a higher melanoma risk with an early start of tanning bed exposure. A cohort study of 73,494 female nurses from the Nurses' Health Study revealed that cancer incidence data over a 20-year span [1989–2009] among who used tanning beds prior to the age of 35 years showed a significantly increased risk of basal cell carcinoma and squamous cell carcinoma (SCC) and a non-significant positive association for melanoma (22).

It has been estimated that more than 400,000 cases of skin cancer may be related to indoor tanning beds in the US (26,27). These cancers have led to 245,000 basal cell carcinomas, 168,000 SCCs, and 6,000 melanomas. According to the data from the 2013 Youth Risk Behavior Surveillance System, many teens used indoor tanning, including 13% of all high school students, 20% of high school girls, 27% of girls in the 12th grade, and 31% of white high school girls (28). Indoor tanners tended to be young, non-Hispanic white (NHW) women (29). A closer look at the findings from the 2010 National Health

Interview Survey showed the following rates of indoor tanning among NHW women: 32% of those aged 18 to 21 years, 30% of those aged 22 to 25 years, 22% of those aged 26 to 29 years, and 17% of those aged 30 to 34 years (29).

Another meta-analysis combining populations from North America, Europe and Oceania, with 14,956 melanoma cases and 233,106 controls concluded that tanning bed use is associated with a subsequent melanoma diagnosis and exposure from more than ten tanning sessions is most strongly associated with the odds of melanoma (15). Although it has been hypothesized that newer models of tanning beds are safer, this study found no statistically significant difference for the association before and after 2000, suggesting that newer tanning technology is not any safer than older models. Including subjects who never used tanning bed as reference, OR for melanoma associated with ever using indoor tanning beds was 1.16 (95% CI, 1.05–1.28). Similar findings were identified among recent studies with enrollment occurring in the year 2000 onward (OR, 1.22; 95% CI, 1.03–1.45) and in subjects attending more than ten tanning sessions (OR, 1.34; 95% CI, 1.05–1.71).

Role of transcription factor nuclear factor erythroid 2-related factor-2 (Nrf2) on melanoma

UV irradiation, xenobiotics, and thermal stress disturb cell metabolism and lead to the increased reactive oxygen species (ROS) generation and to redox imbalance (30). All the factors that lead to an increase of ROS generation and/or a reduction in the antioxidant capacity subsequently contribute to oxidative stress, which expose the skin cells to the formation and accumulation of irreversible damage. Transcriptional regulation of cytoprotective genes by Nrf2 has been proposed as a molecular defense against skin cancer, in particular melanoma (31). Nrf2 encoding genes constitutively expressed under constant expression under physiological conditions. However, the level of Nrf2 in the cytoplasm is regulated by the formation of a Nrf2-Keap1-Cul3 complex (32). Keap1 binds to Nrf2 and directly inhibits its activity, resulting in simultaneous Nrf2 ubiquitination catalyzed by Cul3. Nrf2 is degraded by the proteasome 26S upon the binding of at least four molecules of ubiquitin. Nrf2 is disassociated from the complex when the cells are in oxidative condition, which leads to the oxidation of cysteine residues in the Keap1 molecule (30,33,34). Free Nrf2 is translocated to the nucleus and forms a complex with a small Maf protein. It is then bound to the DNA as antioxidant response element (ARE) and subsequently initiates the transcription of antioxidant genes (35). Nrf2 cytoprotective action is most relevant to antioxidant enzymes, such as glutathione S-transferase (GST), quinone reductase NAD(P)H(NQO1), glutathione reductase (GR), etc. (36-38). Nrf2 also activates the transcription of non-enzymatic antioxidant protein genes containing the ARE recognition sequence (39,40). In addition, Nrf2 can act as a stimulant of anti-apoptotic proteins from the Bcl-2 family (41,42). The fact that Nrf2 has the ability to control a wide range of antioxidants and anti-apoptotic molecules make Nrf2 a significant factor in the cellular response to oxidative stress, in particular in the skin cells.

Cancer preventive mechanisms of sulforaphane

Sulforaphane is a member of the isothiocyanate family and is abundant in broccoli and broccoli sprouts. Isothiocyanates are sulfur-containing compounds including allyl, benzyl, phenylethyl, isopropyl, and methyl thiocyanate (43,44). They are also widely distributed among cruciferous vegetables, such as cauliflower, cabbage, watercress, and kale. The mechanism of sulforaphane action involves a reduction of the glutathione level, which in turn alters the Keap1 conformation and its inhibitory properties such that the active Nrf2 is released into cytoplasm and enhances the expression of antioxidant enzymes (45). Studies have shown that extract containing sulforaphane reduces the risk of UV radiation-induced carcinogenesis in the murine cell line SKH-1 (46). The extract also resulted in a reduction in tumor weight when given to animals with benign skin tumor (47). In human, volunteers subjected to UV light and treated with sulforaphane showed a decrease in the development of skin erythema (48).

Studies have demonstrated that sulforaphane has many physiological effects including anti-cancer, anti-oxidation, and detoxification, which may be involved in the Nrf2 mechanism (49-54). Sulforaphane was found to inhibit melanogenesis and tyrosinase expression (44). The inhibitory effect of 5 uM sulforaphane on melanogenesis was determined to be equivalent to that of 100 uM arbutin, a tyrosine inhibitor (44). Western blot analysis indicated that sulforaphane suppressed melanogenesis, most likely by modulating tyrosinase protein expression. In addition, sulforaphane induced phosphorylated extracellular signal-regulated kinase (ERK) and inhibited phosphorylated p38. It has been reported that the phosphorylated MAPK family (ERK and p38) controls tyrosinase expression. The

results of this study suggested that sulforaphane inhibited melanogenesis and tyrosinase expression by affecting the phosphorylated MAP kinase family, which might serve as an effective skin-whitening agent.

In living systems, elevated levels of ROS may initiate oxidative stress, which can then affect many intracellular targets including DNA. Sulforaphane has been reported to generate ROS, increase global histone acetylation at the Bax and p21 promoters associated with cell cycle arrest (both G2/M and G1) and induce mitochondria-mediated apoptosis with activation of caspases and the specific PARP cleavage [reviewed in (55)]. In addition, sulforaphane was reported to stimulate pro-apoptotic signaling via transcriptional activation of Ap-1, activation of MAPK and death receptors as well as active suppression of pro-survival signals such as NF-κB activation (56,57). Together, these results suggest that sulforaphane is capable of influencing various targets in melanoma cells. Although its efficiency is generally lower *in vivo* compared to the findings from that of cell lines, sulforaphane also induces mitochondrial, caspase-dependent apoptosis (58,59). Detailed information on specific mechanisms and their possible crosstalk in human melanoma are still unclear. Further investigation proved that in sulforaphane-treated cells, elevated levels of ROS stimulate multiple signaling, including activation of the DNA-damage response pathway, increased p38 activity, and enhanced expression of Bax and Puma proapoptotic proteins (55). Thus, DNA damage after sulforaphane treatment was measured by means of microfluorometric detection of phosphorylated histone H2A.X expression in exposed melanoma cells and samples (55). This histone becomes phosphorylated on serine 139 (also called gamma-H2A.X) as a reaction to DNA double-strand breaks, and its fluorescence is proportionate to DNA damage. Sulforaphane induced DNA damage, which was significant and fully comparable at 12 hours of treatment in both cell lines as well as primary melanoma samples. The direct link between sulforaphane-mediated generation of ROS and observed DNA damage in the model was further verified by the observation that pretreatment of samples with the antioxidant N-acetylcysteine (NAC) significantly reduced the number of cells positive for phosphorylated histone H2A.X. To investigate further cellular response to DNA alterations, the involvement of p53 in treated cells was assessed using a p53 DNA-binding assay which measures p53 binding to a specific DNA-response element. The data from this assay showed a time-dependent increase in p53-DNA binding following sulforaphane treatment which

was fully comparable in Bowes and SK-MEL-28 cells as well as in melanoma samples, up to 36 hours of treatment. While at 48 hours of exposure, p53-binding activity peaked in exposed cells and samples there was nevertheless a significant difference between melanoma cell lines versus melanoma samples. While p53-DNA binding differed little between wild-type p53 Bowes cells and mutant p53 SK-MEL-28 cells, a significantly lower p53 activity was detected in melanoma samples.

Discussion

Although melanoma is not as common as non-melanoma skin cancers, the continuously increased incidence rate among Caucasian women in the US is alarming. A proportion of this cancer can be attributable to the use of tanning bed. In recent years, stricter laws and taxes have been put into place to discourage and impede the use of tanning beds, particularly in younger women, or to at least lessen their occurrence. For example, California, Delaware, Hawaii, Illinois, Louisiana, Minnesota, Nevada, New Hampshire, North Carolina, Oregon, Texas, Vermont, Washington, and some cities and counties have banned indoor tanning by minors younger than 18 years (60). However, the use of tanning bed remains popular among certain groups of young females. Additional preventive strategies may need to be explored to reduce the societal burden of this potentially deadly disease. Sulforaphane is a compound found in broccoli extracts and other cruciferous vegetables, and is widely available as a dietary supplement. The safety and lack of toxicity of sulforaphane have also been demonstrated (61). Given the potential biological mechanisms and demonstrated effects of sulforaphane in inhibiting melanoma carcinogenesis, chemopreventive strategies that include sulforaphane present an excellent opportunity to further investigate the mechanistic preventive pathways for melanoma. At the same time, studies employing sulforaphane as dietary supplement may offer a way to prevent melanoma for those who are not voluntarily exposed to high level and long period of sunlight.

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S948

Su et al. Sulforaphane in melanoma prevention from tanning bed UV rays

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S950

Su et al. Sulforaphane in melanoma prevention from tanning bed UV rays

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