The Challenge of Melanoma Chemoprevention

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ABSTRACT

Melanoma is a treatment-resistant cancer of melanocytes. There is a serious unmet need for chemopreventive agents that can inhibit their evolution from pre-existing dysplastic nevi. Low-dose aspirin and non-steroidal anti-inflammatory drugs are potential chemopreventive candidates because they inhibit the enzyme cyclooxygenase-2 which has a number of pro-carcinogenic effects. Unfortunately, the clinical trial reported by Okwundu et al in this issue of Cancer Prevention Research did not show an effect of aspirin on biomarkers associated with progression of pre-malignant dysplastic nevi to melanomas. Further clinical trials with other aspirin or NSAID biomarkers or clinical trials with other potential chemopreventive agents offer hope to those who are at increased risk for melanomas.
Melanoma is an aggressive, treatment resistant cancer of melanocytes. In the U.S. alone, >101,000 new invasive melanomas and 7,180 deaths are estimated to occur in 2021. In most other malignancies, the incidence has either stabilized or declined; in melanoma, they continue to rise. Melanomas often begin as benign nevi, some of which progress over long periods of time to become invasive and metastatic melanomas. The risk factors for melanoma are well defined and include exposure to ultraviolet radiation, (intermittent high intensity sun exposure, use of tanning beds and psoralen photochemotherapy); physical characteristics many of which are associated with polymorphisms in the melanocortin-1 receptor (\textit{MC1R}) (i.e. fair skin and freckling, blue eyes, red hair, and inability to tan), individuals with large numbers of melanocytic nevi, and those who have previously been diagnosed with a melanoma. There are families who are prone to multiple melanomas and in these individuals the melanomas often begin at a young age. Individuals from these families usually have >50 nevi, many of which have atypical clinical and pathological features. Up to 40% have germline mutations in the CDKN2A gene. Thus, there is a large population of individuals for whom chemopreventive agents would be valuable. Because of this and the clinical importance of the problem, there has been great interest in identifying methods for deterring or delaying premalignant nevi from evolving into melanomas (1).

A barrier to melanoma chemoprevention is the difficulty in identifying appropriate calibrated endpoints to evaluate the efficacy of test agents. It is difficult to determine which pigmented lesions will ultimately progress from dysplastic nevi into melanomas based on clinical appearance. Screening for potential chemopreventive agents relies on predictive biomarkers and reliable animal models that mimic the progression of melanoma from melanocytic nevi. Unfortunately, there are few generally accepted biomarkers and only a small number of animal models to use. Changes in histopathological features of dysplastic nevi have proven to be unreliable as biomarkers because of variability in interpretation among dermatopathologists.
Increases in Signal Transducer and Activator of Transcription 3 (STAT3) expression and confocal microscopy have been proposed as potential biomarkers (1). There are several mouse melanoma models, but they are largely restricted to transplantable syngeneic melanoma lines or transgenic mice with enforced expression of mutant oncogenes (N-ras, p16/INK4a, HGF, BRAF, PTEN KO, etc.). While they are important for assessing potential treatments and conducting mechanistic studies, genetically engineered models rapidly develop melanomas without prior evidence of having been dysplastic nevi, making it difficult for prevention studies of pre-neoplastic nevi that progress to invasive melanomas. In a mouse model we have developed, topically applied dimethylbenz(a)anthracene, a polyaromatic hydrocarbon, followed by repeated exposure to 12-O-tetradecanoylphorbol 13-acetate (TPA) or UVA radiation, results in spontaneous nevus formation. Approximately half progress to melanomas whereas the other half regress (2). The melanomas accurately recapitulate many of the molecular changes found in humans and may therefore be a potential animal model to use.

Since ultraviolet radiation exposure is a contributing factor for most melanomas, sunscreens are strongly recommended for people at risk. Clinical information supports their efficacy. In a study in which people in Australia were treated with an Sun Protection Factor (SPF) 15 sunscreen for 5 years and compared to individuals who only used them sporadically, there was a 50% reduction in melanoma incidence in the regular sunscreen group (3). This was statistically significant for invasive melanomas, but not for melanomas in situ. Also, in a prospective population-based study of Norwegian women aged 40-75 years and followed for a mean of 10.7 years, those who applied high SPF (≥15) sunscreens had an 18% reduction in melanoma incidence compared to those who used low SPF (<15) sunscreens (4). Despite the benefits of sunscreens, there is inconsistent patient compliance with their use, large amounts of sunscreen are required to achieve the full SPF value on a product label, and there is no effect of...
sunscreen on prior UV damage. Notwithstanding, the incidence of melanoma has continued to rise over the past several decades even with their use.

Several agents have been examined for melanoma prevention with negative results or potential safety issues (1). These include statin lipid lowering agents that inhibit farnesylation of proteins in the ras signal transduction pathway; selenium, metabolites of which render melanoma cells more susceptible to apoptosis and proliferation inhibition in vitro; and N-acetylcysteine, which has antioxidant effects.

Aspirin (ASA) and non-steroidal anti-inflammatory drugs (NSAIDs) have been attractive candidates for melanoma chemoprevention. They have already been shown to have chemopreventive activities in keratinocyte carcinomas (KCs) and other malignancies (5,6). ASA and NSAIDs inhibit the enzyme cyclooxygenase-2 (COX-2) which is necessary to produce prostaglandin E2 (PGE2). PGE2 has several pro-carcinogenic activities. It is pro-inflammatory, stimulates cellular proliferation and angiogenesis, promotes epithelial mesenchymal transition and regulates anti-tumor immunity. ASA is particularly appealing because is cardioprotective and is not associated with increased major adverse cardiovascular events. The Grossman laboratory has carefully investigated ASA as a potential melanoma chemopreventive agent (7-9). Using melanoma cell lines, ASA significantly reduced colony formation and cell motility and diminished UV-induced DNA damage, both cyclobutane pyrimidine dimers and 8-hydroxydeoxyguanosine. In vivo studies employed TpN61R transgenic mice have melanocyte-specific somatic gene mutations in NRas and Cdkn2a (p16INK4a). These mice develop melanomas after a single neonatal exposure to UV radiation. Treatment with ASA in utero and after birth inhibited UVB-induced PGE2 concentrations in skin and plasma. Although ASA did not reduce the number of tumors or increase their latency, it did inhibit tumor growth rates and DNA damage. These antagonistic effects of ASA on parameters associated with
melanomagenesis were the impetus to a clinical trial examining the chemopreventive activity of ASA in melanocytic nevi.

In the report in this issue of *Cancer Prevention Research*, Okwundu et al, conducted a randomized placebo controlled clinical trial examining ASA on biomarkers associated with progression of dysplastic nevi to melanoma (8). Ninety-five subjects were randomized to receive 81 mg ASA, 325 mg ASA or placebo for one month. One nevus was exposed to solar simulated radiation. One day later the nevus was removed and examined for markers of inflammation and DNA damage. There were no significant differences in T-cell or macrophage populations or in numbers of nevus cells with cyclobutane pyrimidine dimers and 8-hydroxydeoxyguanosine. While this was a setback for melanoma prevention, a different protocol or examination of other biomarkers might offer a pathway for further investigation of ASA and NSAIDs.

Other potential chemopreventive agents for melanoma are being investigated. (Table 1) (1). Nicotinamide, a member of the B3 group of vitamins, is a potentially interesting compound. This agent augments DNA damage repair and in phase 3 clinical trials, it protects high risk individuals against BCC and SCC. Experimental data suggest that it may be useful for melanoma chemoprevention as well (10).

Melanocytes express MC1R, which activates a pathway that stimulates eumelanin synthesis. Pharmacological MC1R agonists have received regulatory approval in some countries for photoprotection in erythropoietic protoporphyria. By increasing pigmentation in skin, MC1R agonists may retard progression of dysplastic nevi to melanoma. Another approach to augmenting pigmentation is administration of Salt Inducible Kinase (SIK) inhibitors, which increase melanin production in animal models. Clinical trials have not yet been initiated and there is a theoretical concern that activating this pathway in dysplastic nevi or melanomas could increase their progression or growth.
Vitamin D3 has potent anti-melanoma activities, and defects in vitamin D signaling contribute to melanomagenesis (11). Vitamin D3 hydroxyderivatives attenuate tumor initiation and promotion by its antioxidant activities, its ability to stimulate DNA damage repair, retard angiogenesis and restrain inflammation. Low serum 25-hydroxyvitamin D3 levels are associated with thicker tumors and poorer prognosis. Decreases in vitamin D receptor and CYP27B1 (an enzyme activating 25-hydroxyvitamin D3) expression are associated with increased aggressiveness of melanocytic lesions. Studies examining vitamin D levels and intake on melanoma have been unrewarding and larger doses of vitamin D have the side effect of hypercalcemia. Non-calcemic analogues of vitamin D also have activities that protect against melanoma and may have potential for melanoma chemoprevention.

There is an inverse relationship between vitamin A consumption and melanoma risk in large case control studies, although the data on vitamin A and its precursor β-carotene in clinical trials have been conflicting (1). In one clinical trial, clinical and histological improvement in dysplastic nevi was observed following topical application of all trans-retinoic acid. Although no improvement was observed with oral isotretinoin, other topical and systemic retinoids are being synthesize which may have potential.

Phytochemicals are being investigated as possible chemopreventive agents for melanoma and non-melanoma skin cancer. Depending on the specific phytochemical, their actions include an antioxidant effect, promotion of DNA damage repair, and inhibition of photoimmunosuppression. Randomized clinical trials have not yet been conducted with these agents for melanoma.

Identification of agents that prevent the progression of dysplastic nevi to melanoma has been challenging. Further studies with ASA examining other biomarkers and clinical trials with the pipeline of other potential agents may ultimately meet the hopes and expectations of individuals at risk for melanoma for a medication that will prevent this serious disease.
REFERENCES


### Table 1
Potential Agents for Melanoma Chemoprevention

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 Endonuclease V</td>
<td>DNA Damage Repair</td>
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<tr>
<td>MC1R agonists</td>
<td>Increase Pigmentation</td>
</tr>
<tr>
<td>SIK Inhibitors</td>
<td>Increase Pigmentation</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>DNA Damage Repair</td>
</tr>
<tr>
<td>ASA</td>
<td>Inhibit DNA Damage, PGE2 Inhibition</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>PGE2 Inhibition</td>
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<tr>
<td>Retinoids</td>
<td>Proliferation Inhibitors</td>
</tr>
<tr>
<td>Vitamin D Analogues</td>
<td>Antioxidant activities, DNA damage repair, Inhibit angiogenesis, Inhibits inflammation</td>
</tr>
<tr>
<td>Phytochemicals</td>
<td>Increase Pigmentation, Antioxidants, DNA Damage Repair, Reverse photoimmunosuppression</td>
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