



Targeting mutant p53 shows promise for sunscreens and skin cancer

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Chronic exposure to UV light is a risk factor for skin cancer in which signature mutations in the p53 tumor suppressor gene occur due to DNA damage and contribute to cancer development. In this issue of the JCI, Tang et al. report on their study of a nonimmunodeficient mouse model of UVB-induced skin cancer and human skin carcinoma cells and show that the mutant p53 conformation-modifying drug CP-31398 not only treats these tumors but also prevents them (see the related article beginning on page 3753). These studies have important implications for chemoprevention as well as therapy of common, mutant p53-driven tumors.

According to the American Cancer Society (1), most of the more than 1 million cases of nonmelanoma skin cancer diagnosed yearly in the United States are considered to be sun related. Nonmelanoma skin cancer, which is the most common type of cancer affecting humans, occurs in either basal cells or squamous cells, and cancers typically occur in sun-exposed areas. Most skin cancers are caused by UV light exposure of the skin to sunlight or man-made tanning lamps (1). There is strong epidemiologic evidence supporting a relationship between UV light exposure and nonmelanoma skin cancer and growing evidence of a relationship between indoor tanning and melanoma (2).

Signature p53 mutations in UV light-induced skin cancer

Although the p53 tumor suppressor gene is widely mutated in cancer and exposure to UV light has been associated with p53 mutations in the skin (3), mutant p53 has yet to be exploited as a therapeutic target in the clinic. UV exposure causes characteristic cyclobutane pyrimidine dimers and 6-4 photoproducts leading to “signature mutations,” such as CC to TT, in hot spots within the p53 gene. Interestingly, UV light-induced tandem CC to TT mutations in the p53 gene are particularly common in transplant patients receiving the immunosuppressant drug cyclospo-

rine (4). In humans, p53 mutations have been associated with squamous cell as well as basal cell carcinoma (5, 6). Unlike humans, who develop squamous cell or basal cell cancers, mice develop only squamous cell carcinomas of the skin following UV light irradiation. Interestingly, the type of mutation or particular hot spot leading to loss of function in p53 has not been associated with aggressiveness of these skin cancers, suggesting the likely involvement of additional events in tumor progression. While mice do not develop basal cell carcinomas following UV light exposure, these tumors do develop in patched heterozygous knockout mice and are enhanced by UV light or ionizing radiation exposure (7).

As a transcription factor, p53 has not presented drug developers with a prototypical drug target. Most current drug development strategies target enzymatic activities: a candidate drug fits into a ligand-binding pocket of a molecule critical for catalysis and serves as an inhibitor. Other popular therapeutic drug targets in cancer are cell-surface receptors that can be targeted by agonist or antagonist antibody therapeutics. Mutant p53 presents a problem for small molecule therapeutic development: as p53 is a transcription factor, it is a challenge to restore p53's wild-type function when the gene becomes mutated, and as p53 is also a nuclear protein, the therapeutic agent has to cross several membranes to reach this nuclear target. Recent proof-of-principle experiments in mice have demonstrated that genetic restoration of wild-type p53 pro-

tein leads to tumor regression (8). Thus, mutant p53 is an important target for cancer therapeutic development because mutations in this “guardian of the genome” not only lead to tumor development and progression but are associated with poor response to therapy (9). It has previously been shown that application of sun protection factor (SPF) 15 sunscreens prior to UV irradiation can virtually abolish p53 mutations in mouse skin and that the inhibition of p53 mutations that are an early event in UV light-induced carcinogenesis might provide a useful measure of protection from skin cancer development (10). Therapy for superficial nonmelanoma skin cancer involves surgical excision as well as topical treatment with 5-fluorouracil or imiquimod (11). The newer, noninvasive options for nonmelanoma skin cancers, including topical chemotherapy, biological immune-response modifiers, retinoids, and photodynamic therapy, may be particularly useful for patients with superficial tumors (12).

CP-31398: a small molecule that restores a wild-type conformation to mutant p53

CP-31398 was reported in 1999 as a small molecule capable of restoring a wild-type epitope (the 1620 epitope) to mutant p53 protein, and this was associated with activation of p53-dependent transcription and antitumor effects in mouse xenograft models (13). It was later shown that CP-31398 can also stabilize wild-type p53 through a pathway that involves reduced p53 ubiquitination with no evidence of p53 amino-terminal phosphorylation typically associated with the checkpoint kinase response to DNA damage (14). CP-31398 has not yet been tested in human clinical trials even though it appears to effectively restore wild-type p53 function to multiple p53 mutants that promote cancer (13, 15). It has not been possible to document stable physical association between CP-31398 and the p53 protein,

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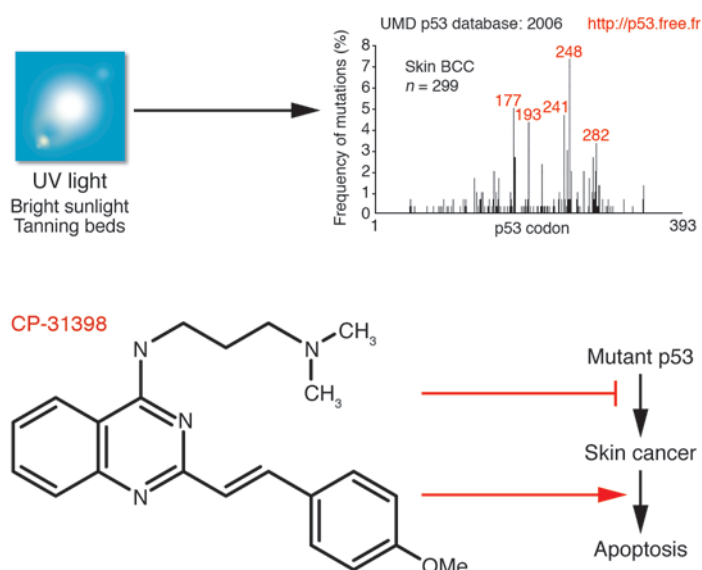


Figure 1

Targeting mutant p53 to prevent or treat UV light-induced skin cancer. UV light exposure from bright sunlight or tanning beds leads to p53 mutations that contribute to skin cancer development in most of the 1 million individuals diagnosed with nonmelanoma skin cancer in the US each year. In a report in this issue of the *JCI*, Tang et al. (17) demonstrate that CP-31398 (13) can prevent UVB-induced tumor development as well as serve as an effective treatment for tumors that develop in an immunocompetent mouse model. CP-31398 appears to promote apoptosis by restoring wild-type p53 activity to mutated p53, leading to increased proapoptotic Bax expression, reduced antiapoptotic Bcl2 expression, and cytochrome c release from mitochondria. The inset shows the spectrum of p53 mutations observed in human squamous cell cancer of the skin that is associated with UVB exposure (UMD p53 mutation database: http://p53.free.fr/Database/p53_cancer/p53_skin.html). Mutations occur at particular hot spots with greater frequency, leading to loss of p53 tumor suppressor function. BCC, basal cell carcinoma.

although restoration of the 1620 epitope, activation of p53 downstream target genes (16), and antitumor effects have been confirmed. Thus, while its physical target has remained elusive, the fact that CP-31398 restores function to mutant p53 proteins has not been disputed. This history and these facts set the stage for further development of this promising anticancer agent.

CP-31398 can prevent and treat UV light-induced squamous cell cancers in mice

In this issue of the *JCI*, Tang et al. (17) have convincingly demonstrated that CP-31398 can effectively treat UV light-induced non-melanoma skin cancers in immunocompetent mice and that the drug can even prevent the development of the tumors (Figure 1). The authors document that CP-31398 increases levels of p53 protein and the p53 target p21 when combined with UVB exposure, over and above what is observed with UV light exposure alone.

CP-31398-treated UVB-exposed skin (but not CP-31398-treated UVB-exposed p53^{-/-} skin) harbored much greater levels of apoptosis than UVB-exposed skin, and UVB-induced tumors had greater levels of the proapoptotic protein Bax and reduced levels of the antiapoptotic protein Bcl2. The authors further documented effects of CP-31398 on mitochondrial localization of p53 and associated changes in membrane permeability. One comment here is that the authors could have done a little more to dissect the relative contribution of the mitochondrial localization of p53 versus its transcriptional response in terms of order of events and requirement for cell death, despite inhibition of one or the other activity. The findings raise a number of issues that are worth further consideration. What mutations in p53 actually developed, and is it the case with severe or prolonged UVB exposure that there are multiple coexisting mutations in different cells destined to become cancerous? If so, which of these could be suppressed by CP-31398 and

which might be more refractory? Is it clear that mutant p53 really is the target for CP-31398 in skin cancer prevention and therapy? While p53^{-/-} mice develop lymphomas and die of them quickly, it is possible to use other p53-deficient models where p53 deficiency is conditional (18). It would then be possible to UV irradiate mice with and without conditional inactivation of p53 prior to UV exposure in order to demonstrate that when p53 is absent, there is no benefit from CP-31398 cancer prevention or therapy. Tang et al. found that patched heterozygous knockout mice that spontaneously develop rhabdomyosarcomas were unaffected by CP-31398 treatment (17). These tumors carried no p53 mutations, further suggesting that mutant p53 was the target in the UVB-irradiation experiments. It would be of interest to determine whether CP-31398 might have an impact on basal cell carcinomas that develop in UVB-irradiated patched heterozygous knockout mice, as they do develop p53 mutations (7).

Targeting mutant p53 in cancer prevention and therapy

The current work reported by Tang et al. is an elegant study that suggests CP-31398 topical application may be active in skin cancer prevention following UV light exposure and may provide an effective therapy after cancer development (17). There are some other questions that arise from this work: Does CP-31398 prevent UV light-induced skin cancer development by restoring the 1620 epitope (wild-type p53) to mutated p53 or by limiting propagation of damaged cells by activation of the remaining wild-type p53 allele prior to its loss in skin cancer development? How does topical CP-31398 compare with topical 5-fluorouracil or topical imiquimod in the treatment of UVB-induced skin cancers? Can topical CP-31398 prevent melanoma that has some association with UV light-induced damage, although p53 mutations are uncommon? In this regard, a recent study found that an organometallic glycogen synthase kinase 3- β inhibitor is a potent activator of (wild-type) p53 and an inducer of cell death in highly chemoresistant melanoma cells (19). Can CP-31398 be added to sunscreen, and if so, what are the short-term and long-term side effects on human skin, given that CP-31398 can also stabilize and activate wild-type p53, albeit without evidence for DNA damage (14)? Other approaches in the evolution of sunscreens include use of sulforaphane-con-



taining chemical extracts from broccoli that boost production of protective enzymes in skin and protect from UV-induced erythema and inflammation in humans (20). With respect to cancer prevention, it is worth asking whether systemic CP-31398 might prevent cancer development in patients with Li-Fraumeni syndrome – a rare autosomal dominant hereditary disorder in which patients possess a mutation in the p53 tumor suppressor gene that greatly increases their susceptibility to cancer. The study by Tang et al. (17) reports exciting progress with clinical relevance and, like all outstanding papers, raises interesting questions for future work. Further studies and clinical translation of the findings of Tang et al. may lead to improved ways of preventing and treating UV light-induced skin cancers that afflict millions of people.

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IL-6 involvement in epithelial cancers

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In this issue of the JCI, two reports provide intriguing new information on the role of the inflammatory cytokine IL-6 in breast and lung cancer. The study by Sansone et al. implicates IL-6 in the instigation of malignant properties in breast cancer stem cells (see the related article beginning on page 3988). The study by Gao et al. identifies mutant variants of EGFR as inducers of IL-6 in lung adenocarcinomas (see the related article beginning on page 3846). These studies add to our understanding of potential roles for IL-6 in cancer and further motivate investigations of IL-6-targeted chemotherapeutics.

IL-6 is a multifunctional cytokine that was originally characterized as a regulator of immune and inflammatory responses; however, elevated expression of IL-6 has been detected in multiple epithelial tumors (1). IL-6 binds to a heterodimeric receptor,

which contains the ligand-binding IL-6 α chain and the common cytokine receptor signal-transducing subunit gp130. IL-6 receptor engagement leads to activation of the JAK family of tyrosine kinases, which then stimulate multiple pathways involving MAPKs, PI3Ks, STATs, and other signaling proteins (2).

Given the reported involvement of IL-6 and its downstream targets in the regulation of cell proliferation, survival, and metabolism, it is not surprising that IL-6 signaling has also been implicated in

tumorigenesis (3). However, the nature of IL-6's involvement in cancer has been quite controversial, as dichotomous roles for IL-6 in both tumor-promoting and -suppressive activities have been reported. For example, IL-6 signaling has been linked to both pro- and antiapoptotic activity in breast cancer cells (4, 5). Multiple studies have documented high IL-6 levels in the serum of patients with certain carcinomas (i.e., breast, lung, lymphoma) and have correlated high IL-6 levels with a poor clinical prognosis (2). These data imply an oncogenic role for IL-6; however, lacking is an understanding of the mechanisms governing IL-6 production in tumors and the biological role of this cytokine in tumorigenesis. Two reports in this issue of the JCI (6, 7) advance our understanding of both of these issues and provide a molecular rationale for the development of anti-IL-6 therapeutics (summarized in Figure 1).

Nonstandard abbreviations used: CA-IX, carbonic anhydrase IX.

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