Skin problem. There are a variety of human skin types, but pale people who don't tan seem to have the least protection from sun-induced skin cancers, including deadly melanomas.

SKIN BIOLOGY

A Healthy Tan?

A dark natural tan offers unparalleled protection against skin cancer. So scientists are developing compounds that trigger tanning without the sun's damaging effects

Anyone who relies on sunscreen knows it is sticky, inconvenient, and easy to forget. But sunscreen has a lesser known, and more serious, downside: It doesn't adequately protect against the deadliest form of skin cancer.

Although ultraviolet (UV)-blocking sprays and creams protect people against sunburn and the milder forms of skin cancer—squamous cell and basal cell carcinoma—they do not form an effective shield against melanoma, which doctors diagnose in 132,000 people worldwide each year. Ironically, says a growing cadre of skin biologists, what seems to protect best against melanoma is something that sunscreens efficiently thwart: a deep, dark tan.

Dark-skinned people, who also tend to tan well, are up to 500 times less likely to get melanoma and other skin cancers than are fair-skinned individuals. The ability to tan confers protection, researchers say, regardless of the skin's background level of pigmentation. This is due in part to the UV-shielding effect of melanin, the pigment that makes skin cells dark, and perhaps in part to an acceleration of DNA repair that some believe accompanies tanning. But tanning in the sun is a fool's wager, dermatologists say, because it causes dangerous DNA damage, which may lead to cancer before it can be fixed. To provide a sun-independent alternative, scientists are now developing compounds that trigger tanning and DNA repair by acting on molecules that control the melanin production pathway.

One key molecule is the melanocortin 1 receptor (MC1R), a protein on the surface of melanocytes that heads a major tanning pathway. Some researchers are targeting MC1R directly to stimulate tanning, whereas others are bypassing it and aiming at downstream targets in that pathway—a strategy that could help fair-skinned people who have mutations in the receptor's gene and thus normally don't tan (see sidebar, p. 1215). Still other investigators are concocting skin-cancer preventatives that promote MC1R-independent DNA repair within the skin, in some cases while also promoting melanin production.

"We hope to develop something that works far better than a sunscreen," says pigment cell researcher Zalfa Abdel-Malek of the University of Cincinnati College of Medicine in Ohio, who is developing an MC1R stimulator. "It will allow your pigment cells to make melanin and protect themselves against subsequent sun exposure." Adds pediatric oncologist David Fisher of Harvard Medical School in Boston, who is aiming elsewhere in the same pathway: "By switching on pigmentation, we may be able to mimic the epidemiological groups that have the lowest risk of melanoma," which are people with dark skin or who tan easily.

None of the candidate tanning compounds or DNA-repair agents has yet been proven safe and effective in large numbers of people. And there remain some who question the cancer-protective aspects of tanning alone, noting that tanned skin has a sun protection factor (SPF) of just 2 to 4. These skeptics argue that dark-skinned people may have other physiological features that protect them from skin cancer and that a pale person with an artificially induced tan may enjoy minimal cancer protection. "Even if you increase tanning, the improvement in photoprotection is likely to be small," suggests dermatologist Jonathan Rees of the University of Edinburgh, U.K.

Tanning tales

Cosmetic companies as well as researchers have been experimenting with skin-darkening agents for decades. There are many tanning agents on the market today, but they only dye the skin without engaging the natural tanning process or protecting the skin from UV rays.

The first scientific step toward a true artificial tanning agent came in the 1960s, when Yale University dermatologist Aaron Lerner discovered that injecting people with crude extracts from the hypothalamus containing the newly discovered melanocyte-stimulating hormone (MSH) increased skin pigmentation. Then in 1991, a team led by biologist Mac Hadley of the University of Arizona, Tucson, reported that injecting a long-lived analog of MSH increased skin pigmentation without sun exposure in 28 Caucasian men.

But no one understood how MSH acted on skin cells. In 1992, Roger Cone and his colleagues at Oregon Health Sciences University in Portland reported cloning the hormone's receptor, MC1R, in humans and mice. They also showed that mutations in that receptor gene underlie varying coat colors in mice: If mice inherited two defective receptors, they were yellow, whereas mice with at least one

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highly efficient MC1R protein were black or partially black. In 1995, Rees and several colleagues reported a similar association between aberrant forms of the receptor and variations in skin and hair color in people.

But it wasn't clear until recently that MSH and MC1R play an integral role in the skin's natural tanning response. In a report in the 21 September 2006 issue of *Nature*, Fisher, along with John D'Orazio, then a postdoc in Fisher's lab at the Dana-Farber Cancer Institute in Boston, and other colleagues, proved that connection. They studied a mouse that, like redheaded people, has two defective copies of the gene for MC1R. Unlike mice with working receptors, the pink-skinned "redheaded" mice could not tan at all, showing that a functioning MC1R is necessary for the process, at least in rodents.

In cell culture experiments, the group demonstrated that UV radiation prompts the release of MSH from keratinocytes, the dominant cell type in skin. The MSH then triggers MC1R on melanocytes, which produce melanin after a cascade of chemical reactions that begins with the activation of the enzyme adenyl cyclase, yielding an upsurge in cyclic adenosine monophosphate. Once melanocytes transfer the melanin to keratinocytes, the pigment forms caps over cell nuclei, shielding their DNA and creating the skin's tanned look. The pigmented keratinocytes protect the melanocytes below them as well.

Activation of the tanning pathway by MSH also seems to initiate DNA repair. Skin biologists Markus Böhm and Agatha Schwarz of the University of Münster in Germany and their colleagues reported in 2005 in the *Journal of Biological Chemistry* that the application of MSH reduced amounts of cyclobutane pyrimidine dimers, a sign of DNA damage, in cultured melanocytes exposed to UVB rays. These results and others indicating an enhanced DNA repair ability in tanned skin may explain its protective capacity beyond its simple SPF. "If you are genetically blessed with skin that tans well, only part of that is the melanin; you also have a repair mechanism that jumps to the challenge," says Barbara Gilchrest, a dermatologist at Boston University (BU) School of Medicine. "When you tan, you increase the level of DNA repair proteins by a factor of 2 or 3."

Protective potions

An agent targeting MC1R or other molecules in the tanning pathway might confer both advantages: the protection provided by pigment production and better DNA repair. For example, scientists at Clinuvel Pharmaceuticals, based in Melbourne, Australia, are testing a slow-release formulation of one of Hadley's injected MSH analogs, with the idea of initially using it as a preventive treatment for various sun-related ailments such as the common sun rash called polymorphous light

Why I Have Red Hair, Need to Avoid the Sun, And Shouldn't Commit a Crime

I am a redhead who cannot tan, and so are my two children. My husband, on the other hand, has dark brown hair and tans reasonably well. Surprisingly, red hair and the inability to tan are largely endowed by variations in a single gene: one for a receptor on the surface of melanocytes dubbed MC1R. Epidemiologists have discovered about 75 alleles for the *MC1R* gene, a handful of which disrupt the function of the receptor. In 1995, Jonathan Rees of the University of Edinburgh, U.K., and his colleagues reported that more than 80% of the people with red hair or fair skin they tested had such defective *MC1R* alleles. By contrast, these versions of the gene were present in fewer than 20% of study participants with dark hair and in less than 4% of those who tanned well.

Since then, larger studies, including one by Richard Sturm at the University of Queensland in Australia, have confirmed the association between faulty *MC1R* alleles and light skin and red hair. Redheads like me almost always have two alleles encoding defective MC1R proteins.

As part of my reporting on artificial tanning agents (see main text), geneticist Greg Barsh of Stanford University in Palo Alto, California, agreed to help me learn my *MC1R* genotype. Barsh's postdoc, Linda Ste. Marie, identified what appear to be two different alleles for a malfunctioning receptor, suggesting that I am a so-called compound heterozygote. One of these alleles, known as R151C, is fairly common, appearing in 10% to 20% of people with European ancestry. It is caused by a single-nucleotide exchange that inserts the amino acid cysteine in place of the usual arginine at codon 151. The result is that cells produce fewer of the receptor or it has diminished function, or both.

Barsh had never heard of my other allele, in which a single-nucleotide swap at position 456 produces a genetic stop sign that would halt MC1R's manufacture early. A receptor that is missing half of its amino acids can hardly be expected to work.

In 2000, Sturm's group reported that any of three alleles associated with red hair, including my R151C, double a person's risk of melanoma. To me and other redheads, this is not a big surprise, because dermatologists have already shown that our typical pale skin color is a reliable predictor of increased skin cancer risk.

CREDIT: VICTORIA VERNON

More important, *MC1R* status may help size up melanoma risk in people who do not have the physical characteristics associated with that greater cancer threat. In the 28 July 2006 issue of *Science* (p. 521), Maria Teresa Landi of the U.S. National Cancer Institute in Bethesda, Maryland, and her colleagues reported that possessing just one allele for a poorly functioning MC1R raises the risk of a dangerous type of melanoma more than threefold in people who have darker skin or hair. My husband may be one of these people, because my redheaded children presumably received one of their inactive MC1R proteins from their father.

MC1R genotype might also inform decisions about which of the experimental tanning compounds could work best. In theory, MSH analogs might not be the choice in people like me with defective MC1R receptors. Mysteriously, however, redheads have responded with tans in trials of such drugs. Spelling out a person's *MC1R* genes could also help crime-scene investigators, Rees suggests. If the analysis of biological tissue left at the scene reveals two aberrant versions of *MC1R* alleles, there is a 90% chance that its owner has red hair. That little fact should keep me on the straight and narrow. **–I.W.**

eruption. In the August 2006 *Journal of Investigative Dermatology*, a team led by dermatologist Ross StC. Barnetson of Royal Prince Alfred Hospital in Camperdown, Australia, reported that three 10-day cycles of the Clinuvel treatment increased skin melanin content by 41% in 47 healthy, fair-skinned people, preventing them from sunburn and significantly reducing signs of DNA damage in their skin. The drug will soon be tested in fair-skinned organ-transplant recipients to see whether it reduces the number of precancerous lesions such patients typically develop from the transplant's regimen of immune-suppressing drugs.

Clinuvel's drug must be injected every 2 months to maintain a tan. By contrast, Cincinnati's Abdel-Malek and her colleagues have developed potent MSH analogs small enough that they might be administered topically. Two such peptides stimulated melanin production, reduced programmed cell death, and enhanced DNA repair in melanocytes exposed to UV light, the researchers reported in the July 2006 *FASEB Journal*.

MSH analogs may not work in redheads with two damaged genes for MC1R proteins, however. Other teams are therefore aiming compounds downstream in the MC1R pathway. Fisher, D'Orazio, now at the University of Kentucky College of Medicine in Lexington, and their colleagues created a spectacular artificial tan in their redheaded rodents by smearing the shaved animals daily with the small molecule forskolin, a natural product in some teas that stimulates adenyl cyclase activity in cells. The forskolin-induced tan protected UV-exposed mice against sunburn and the production of DNA adducts, a sign of DNA damage. In redheaded MC1R-lacking mice that also had defective DNA repair enzymes, and thus are prone to UV-induced tumors, forskolin significantly reduced the number of such tumors compared to similar mice in a control group, Fisher says. Fisher has co-founded a firm, Magen Biosciences in Cambridge, Massachusetts, that is now trying to develop drugs that hit molecules more specific to the tanning pathway, because virtually all cells contain adenyl cyclase.

An earful. This mouse's right ear darkened after a compound that triggers the tanning pathway was applied to the skin.

CREDIT: DR. JOHN D'ORAZIO/UNIVERSITY OF KENTUCKY

Damage signal

The MC1R pathway is not the sole arbiter of tanning. In addition to stimulating release of MSH, UV light triggers DNA damage, and many researchers believe that such damage can itself induce tanning and DNA repair by a separate mechanism. Gilchrest theorizes that this DNA-damage response revolves around telomeres, looplike structures at the ends of chromosomes that contain repetitive DNA sequences.

Starting in the 1990s, Gilchrest and her colleagues found that exposing skin cells to DNA fragments with specific sequences triggered both tanning and DNA repair. Gilchrest concluded that the fragments that triggered tanning, which she dubbed T-oligos, were eliciting restorative DNA-damage responses in healthy skin cells by imitating the exposed end of damaged telomeres that had lost its loop structure.

In a recent study reported in the September 2006 *FASEB Journal*, the BU researchers applied T-oligos over 5 days to patches of human skin in culture from 18 Caucasian donors. The treatment boosted the melanin content of the skin samples three- to fivefold, comparable to UV's effects, and greatly accelerated the removal of markers of DNA damage in the skin after exposure to UV light compared to untreated, UV-exposed skin samples from the same donors. The T-oligos also increased levels of the cancer-suppressor protein p53 after UV irradiation. These findings, Gilchrest says, support the idea that sunscreen lotions incorporating T-oligos could produce tans in people, protecting them against sun damage and skin cancer. BU has patented this strategy, and Gilchrest is now trying to get funding for additional animal tests that could pave the way for human trials.

Meanwhile, other researchers are experimenting with lotions that trigger DNA repair without promoting a tan. Scientists at AGI Dermatics in Freeport, New York, have been testing a skin lotion called Dimericine that contains a bacterial DNA repair enzyme, T4N5, packaged into liposomes, microscopic lipid spheres that help cells absorb the enzyme. Molecular biologist and AGI head Daniel Yarosh envisions his product as a "morning-after cream" that could reduce the risk of cancer and other skin problems after a person has spent too long in the sun. Sunburned skin cells ordinarily repair half the DNA damage within 24 hours, whereas Yarosh cites published studies indicating that Dimericine-treated cells eliminate most of the damage within 6 hours.

In 2001, AGI Dermatics reported in the *Lancet* on a study of the lotion in people with a rare disease called xeroderma pigmentosum (XP), in which a lack of DNA repair enzymes leads to very high skin cancer rates. Compared to 10 XP patients who received a placebo lotion, a year of Dimericine treatment in 20 XP patients lowered the rate of precancerous lesions and basal cell carcinomas by 68% and 30%, respectively. (Currently under way are company-sponsored skin-cancer prevention trials in renal transplant patients and people with a history of skin cancer.)

A skin lotion instead of an injected drug like Clinuvel's, Dimericine has displayed few side effects so far in its tests on people, although some could crop up in larger trials. Some experts worry about artificially triggering the tanning response using agents that target players in the MC1R pathway, especially via drugs administered to the whole body and not just the skin. The Clinuvel compound, for instance, has caused nausea and vomiting in study subjects. There are more serious hypothetical concerns as well. "If you add MSH to melanocytes, they divide more quickly" in culture, raising the specter of cancer, Rees says. Then, of course, there's a sociological question of how many pale-skinned people would actually darken their skin to protect themselves against skin cancer.

On the other hand, the incidence of melanoma has tripled in the past 40 years despite the increased use of traditional sunscreens. Given that, D'Orazio, for one, thinks it's worth trying to develop novel ways to protect people against a ubiquitous, known mutagen—that is, UV light.

–INGRID WICKELGREN