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Engineered Skin Offers Clues to Melanoma Development

When it comes to the deadly skin cancer melanoma, studying functional tissue rather than cell lines may better provide insight into the disease's development, according to new research from a Howard Hughes Medical Institute predoctoral fellow at Stanford University School of Medicine.

Though multiple genetic alterations are associated with melanoma development, scientists have not been able to establish a direct causal link between these alterations and human cancer growth. Determining whether these mutations have the potential themselves to induce cancer or simply play a supporting role also has been difficult.

"Our results highlight the importance of moving away from simplified cell transformation studies to studies of functional human tissue."

— **Yakov Chudnovsky**

To determine the impact of genetic alterations associated with human melanoma, Yakov Chudnovsky; his advisor, Paul Khavari; postdoctoral fellow Amy Adams, and colleagues generated human skin tissue containing cells selectively engineered to express specific mutations found in melanoma. They report their findings in the June 12, 2005, issue of the journal *Nature Genetics*, offering clues to the oncogenic potency of several genes implicated in the development of melanoma.

Melanoma is the deadliest form of skin cancer, resulting from the malignant transformation of cells called melanocytes. Over the past four decades, the incidence of melanoma has risen 15-fold, a more rapid increase than that of any other cancer.

"The only current treatment is early detection and excision," Chudnovsky said. "But no treatment can substantially enhance patient survival once metastasis has occurred."

To simulate the environment in which melanoma naturally arises, the research team introduced one or more cancer-associated genes into human melanocytes, the pigment-producing cells that normally reside in the deepest

layer of the skin. The genetically engineered melanocytes were then combined with keratinocytes, the cells that give skin its structure, to form a sample of human skin that was grafted onto laboratory mice. The mice were observed for up to six months and sacrificed at different time points to examine the skin grafts for signs of melanoma and to determine whether the cancer had metastasized.

The researchers began by introducing several mutant genes commonly found in human melanomas. These included genes that interfere with the retinoblastoma (Rb) and p53 tumor suppressor pathways, which normally act to keep cell growth in check, as well as human telomerase reverse transcriptase (hTERT), the enzyme that protects the ends of chromosomes during cell division. This enzyme is associated with the progression of many cancers. None of these mutant genes, when introduced individually or in combination, led to cancerous growth. But when a mutant form of Ras — produced by an oncogene that sends growth signals to a cell—was added to this combination, it produced clinical features of invasive human melanoma: darkly pigmented skin that progressed to ulcerated tumor nodules.

Melanoma was observed as early as one month after the oncogenic combination was introduced. The tumors demonstrated aggressive local invasion but did not metastasize.

Next, Chudnovsky and colleagues investigated whether interfering with either the Rb or p53 pathways could trigger melanoma. They found that in combination with Ras and hTERT, the expression of cyclin-dependent kinase 4 (CDK4), which promotes cell growth and inhibits Rb function, induced invasive melanoma in human tissue. Similarly, inhibition of p53, when combined with Ras and hTERT, resulted in invasive melanoma.

The scientists also investigated the role of elevated telomerase activity. In combination with other cancer genes, hTERT, the active protein in the enzyme telomerase, caused progressively invasive melanoma. In contrast, melanocytes that did not receive hTERT remained in an early stage of benign growth.

Finally, the team looked at the PI3K and Raf pathways, frequently implicated in the development of melanoma. They found the expression of active PI3K, together with CDK4, hTERT, and inhibition of p53, produced invasive melanoma growth indistinguishable from that caused by Ras. By contrast, B-Raf—the most common mutant gene in human melanoma—combined with CDK4, hTERT and inhibition of p53, could not cause full-scale melanoma.

When it comes to melanoma, PI3K and B-Raf often “show up at the scene of the crime,” said Chudnovsky's graduate advisor, Paul Khavari, but it has been unclear “which one is the murderer and which one is the accomplice.” Based on this study, it appears that mutations that activate the PI3K cascade could be a primary pathway leading to melanoma.

“Our results highlight the importance of moving away from simplified cell transformation studies to studies of functional human tissue,” Chudnovsky

said. “Skin is easily accessible and can help us really understand how cancer develops, so we can develop new therapeutic targets.”

Chudnovsky carried out this work in Khavari's laboratory, in close collaboration with postdoctoral fellow and co-first author Adams. The team plans to use the human-tissue model of melanoma to evaluate potential treatments.