Cancer and TCDD: The Mitochondrial Connection

During the Vietnam War, from 1961 to 1971, U.S. military forces sprayed millions of gallons of the herbicide Agent Orange over vast tracts of Southeast Asian jungle, mainly in an effort to remove foliage and expose enemy troops. Troops were exposed to TCDD that contaminated the Agent Orange, and since the 1970s, elevated blood TCDD concentrations have been implicated in many cancers, skin rashes, and other health problems experienced by Vietnam veterans. Although TCDD is carcinogenic, it is not directly genotoxic. A report in the 8 January 2008 Proceedings of the National Academy of Sciences now demonstrates one of the ways that TCDD may promote cancer's growth and spread.

The new study describes a novel mechanism of TCDD action that focuses on the mitochondria: “We found that TCDD induces tumor cell proliferation and invasion by directly acting on mitochondrial transcription machinery and inducing mitochondrial respiratory stress,” says principal investigator Narayan G. Avadhani, a biochemistry professor at the University of Pennsylvania. Such mitochondrial dysfunction inhibits apoptosis in malignant cells and increases the invasive potential of cancer. Mitochondrial dysfunction is also associated with conditions such as heart disease, diabetes, obesity, blindness, deafness, kidney disease, and neurodegenerative disorders, as well as with aging.

[The respiratory stress-signaling] cascade culminates in the activation of a large number of nuclear genes that affect various cellular processes including cell metabolism, proliferation, and apoptosis,” says lead author Gopa Biswas, a researcher in Avadhani’s lab. “We have now established that TCDD alters cellular morphology and physiology through a similar mechanism.”

It is generally accepted that adverse effects of TCDD result from its activation of the Ah receptor, with effects occurring at very low exposures. In the presence of TCDD, the Ah receptor has been shown to either induce or suppress the transcription of numerous genes that have been linked with cancer development via changes in tumor suppressor proteins, oncogenes, growth factors, and cell cycle proteins, among other factors.

Mitochondrial dysfunction may entail a more fundamental mechanism. It appears that TCDD-induced mitochondrial stress signaling in cancer cells is propagated in part through the Ah receptor but also acts through mechanisms that are independent of the Ah receptor, such as by inducing protein kinase C and extracellular signal-regulated kinases.

“Our findings show that at subtoxic levels of ten to fifty nanomolar, TCDD is sufficient to cause mitochondrial dysfunction and induce the signaling cascade,” says Avadhani. “These results raise concerns over the adverse health implications of dioxins and PCBs even at very low levels.”

In both animal and human studies (notably epidemiologic analyses of cancer rates following the 1976 industrial accident in Seveso, Italy), TCDD exposure has increased cancer incidence and mortality at all cancer sites rather than at a few specific sites. In 1997, the International Agency for Research on Cancer upgraded TCDD to a Group 1 human carcinogen on the basis of mechanistic data. Considering subsequent dose–response assessments for TCDD and cancer, Kyle Steenland, a professor of environmental and occupational health at Emory University, and colleagues argued in the September 2004 issue of EHP that “TCDD exposure levels close to those in the general population may be carcinogenic and argue for caution in setting the upper ranges of long-term permissible exposure to dioxins.”

The present study is limited in that it involved skeletal myoblasts, not living organisms. “These findings are significant but unfortunately provide no in vivo data showing tumor progression in animals due to loss of mitochondrial function by TCDD,” says Keshav K. Singh, a cancer geneticist at Roswell Park Cancer Institute in Buffalo, New York. “At a minimum, xenograft studies in mice are needed.” Avadhani now plans to study the precise mitochondrial targets of different polychlorinated biphenyls (a related group of compounds) that lead to reduced mitochondrial transcription and then examine the implications of this pathway in tumor progression in vivo. He sees possible implications for the prevention of breast, pancreatic, and other endocrine cancers.

Recognition that the carcinogenic effects of environmental toxicants may originate in disruption of mitochondrial biology could prove important for the future development of cancer prevention and treatment procedures related to TCDD and other dioxin exposures. “The new findings suggest that the risk of cancer may be reduced by avoiding or lowering exposure to environmental mitochondrial toxicants as well as [possibly] by optimizing mitochondrial energy metabolism by nutritional and medicinal means,” says Egil Fosslien, a pathology professor emeritus at the University of Illinois at Chicago. –M. Nathaniel Mead
**Alternative Test Models**

**Toxicity Testing Takes Stock**

Early February 2008 saw the celebration of the first 10 years of work by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the federally funded body charged with promoting the regulatory acceptance of scientifically valid safety testing methods that replace, reduce, or refine the use of animals. ICCVAM also released a five-year plan establishing priorities for research, translation, and validation activities. The plan was unveiled during an anniversary symposium held 5 February 2008 in Bethesda, Maryland.

ICCVAM conducts technical evaluations of alternative testing methods proposed for regulatory use, makes recommendations to regulatory agencies on the usefulness and limitations of new methods, identifies knowledge and data gaps that need to be addressed with further research and development efforts, and coordinates with similar efforts internationally. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), housed at the NIEHS, provides administrative, operational, and scientific support to the interagency committee.

To date, ICCVAM’s recommendations have resulted in national and international acceptance of alternatives for acute oral toxicity, skin corrosivity, and allergic contact dermatitis, three of the most common types of toxicity assays. ICCVAM has also recommended use of the Bovine Corneal Opacity and Permeability and Isolated Chicken Eye tests to assess eye irritation; regulatory decisions on these assays are due in April 2008.

William Stokes, director of NICEATM, says the tests reviewed by ICCVAM are essential to translating research findings from bench to bedside. “Our ‘bedside’ is more effective public health prevention measures,” he says. “The improved alternatives recommended by ICCVAM help prevent disease and injury so you don’t end up in the bed in the first place.”

“ICCVAM is essential,” says John Bailey, executive vice president for science at the Personal Care Products Council (formerly the Cosmetics, Toiletry, and Fragrance Association). “It brings the science together, and allows a transparent assessment.”

ICCVAM’s new five-year plan lists four areas of emphasis: establishing priorities for future testing; identifying and encouraging appropriate research efforts; educating stakeholders about the acceptance and appropriate use of improved methods; and improving partnerships with and between those inside and outside ICCVAM. Some of the approaches emphasized for further development include high-throughput assays, use of species such as roundworms and tadpoles, and better biomarkers of toxic effects.

Despite the committee’s successes, ICCVAM’s efforts have received mixed reviews. Jessica Sandler, director of the Regulatory Testing Division for People for the Ethical Treatment of Animals, who strongly supported ICCVAM when it was formed, says progress has been painfully slow. She attributes this in part to ICCVAM not taking advantage of the work of sister organizations such as the European Centre for the Validation of Alternative Methods (ECVAM), which has endorsed the use of many more alternative test methods than its U.S. counterpart.

But Stokes explains that ICCVAM, NICEATM, and ECVAM in fact worked very closely in recent years. ICCVAM’s activities are limited to the review of test methods applicable to regulatory testing, while ECVAM has a broader mandate to address animal use for all areas of research and testing (e.g., screening to prioritize chemicals for product development). Stokes notes that ECVAM is a center with numerous laboratories and a large full-time staff, whereas ICCVAM is a committee with no laboratories. ICCVAM must also operate with a high level of transparency and the opportunity for broad public and stakeholder input in contrast to ECVAM’s less-extensive closed review process. Moreover, Stokes says, many of the assays recommended by ECVAM have not been accepted by European regulatory agencies.

Daniel Krewski, chairman of a National Research Council committee that produced the 12 June 2007 report *Toxicity Testing in the 21st Century*, affirms the value of ICCVAM’s work so far. However, the committee recommends that an entirely different federal framework, possibly with a size and budget akin to that of the National Toxicology Program, be rapidly adopted to overcome ICCVAM’s limitations. Such a program would be “quite a paradigm shift from what we’re doing now,” he says, but ICCVAM could be viewed as a transitional step toward this new direction.

Meanwhile, the new five-year plan may help expedite improvements, says Sonya Lunder, a senior analyst with the Environmental Working Group. She has many concerns about the ICCVAM review process but appreciates the plan: “It gives us a benchmark to measure progress.” The plan is available at http://iccvam.niehs.nih.gov/docs/5yearplan.htm. -Bob Weinhold

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**Savory Relief for Arsenic Poisoning?**

In the February 2008 issue of *Food and Chemical Toxicology*, researchers from India report that aqueous garlic extract (AGE) fed to at-risk individuals may reduce the toxic effects of arsenic. Rats receiving daily doses of arsenic equivalent to the levels in groundwater from heavily arsenic-contaminated areas of the Bengal Basin retained significantly less of the element in blood and liver and excreted significantly more in urine when fed 2 mg/mL AGE. The researchers believe the antioxidant properties of garlic, along with the chelating efficacy exhibited, led to the success of the treatment. AGE was also seen to significantly reduce intracellular reactive oxygen species in several cell types.

**In Utero Cigarette Smoke Exposure and Age at Menopause**

It is well documented that women who smoke cigarettes begin menopause earlier than nonsmokers. Using data from a U.S. national study on the health effects of prenatal diethylstilbestrol exposure, a team of researchers reported in the 11 January 2008 advance access edition of the *American Journal of Epidemiology* that study participants who had never smoked cigarettes but had been prenatally exposed to maternal cigarette smoke experienced earlier-onset menopause. Moreover, previously noted associations between current smoking and age at menopause were not observed among these women.

**Antibiotic Resistance Seen in Arctic Wildfowl**

Swedish researchers reported in the January 2008 issue of *Emerging Infectious Diseases* that birds living in three different geographic regions of the Arctic tundra carry *E. coli* bacteria resistant to multiple types of antibiotics. These birds, which lived in northeastern Siberia, northern Alaska, and northern Greenland, are believed to have had no contact with humans. The researchers proposed three possible explanations for their findings: the birds could have been exposed to the bacteria through contact with other species of birds migrating from other regions, or resistance could have developed either through spontaneous mutations or through horizontal gene transfer from other microbes.
Dairy Paradox

The etiology of colorectal disease revolves around genetic and environmental factors, particularly diet. A meta-analysis in the 7 July 2004 issue of the *Journal of the National Cancer Institute* suggested that consuming more dairy products and calcium may reduce colorectal cancer risk, but epidemiologic studies on this link have yielded inconsistent results. One explanation for this inconsistency may be the timing of exposure: cancer develops over decades, and early-life exposures to carcinogens and growth factors could be a critical factor. A new study designed to address this possibility has found that adults who consumed more dairy during childhood may have a greater risk of developing colorectal cancer in adulthood. The results appear in the *American Journal of Clinical Nutrition*.

Colorectal cancer is among the leading causes of mortality in developed countries, and this trend is likely to continue in the future. The number of new cases is expected to increase by 40% between 1996 and 2015, according to the American Cancer Society. The increases are expected to be greatest for colorectal cancer, which is the second leading cause of cancer death in men and the third leading cause of cancer death in women. The main culprits appear to be high-calorie diets and obesity, which are associated with increased risk of colorectal cancer.

The mechanisms underlying the associations between diet and colorectal cancer are complex and not fully understood. However, it is clear that dietary factors play a role in the development of colorectal cancer. Changes in diet and lifestyle may help reduce the risk of colorectal cancer, but more research is needed to understand the underlying mechanisms.

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factor 1 (IGF-1), a key player in the development and progression of colorectal cancer. But it’s the effect of early dairy intake on childhood (rather than on adult) concentrations of IGF-1 that may be the important mediator of colorectal cancer risk.

The findings seem perplexing given previous research showing an association between high dairy/calcium consumption and lower risk of colorectal cancer in adulthood. “In adults, this protection occurs despite the increase in growth factors, which would be expected to increase risk,” says epidemiologist Edward Giovannucci of the Harvard School of Public Health. “It is possible, though not proven, that the increase in growth factors early in life may be more important for colorectal cancer risk.” Giovannucci asserts that the new findings are biologically plausible and warrant efforts to replicate these findings in other populations and settings.

Andrew Szilagyi, a gastroenterologist and assistant professor of medicine at McGill University School of Medicine, points out the lack of data on adult dietary intakes in the Boyd Orr cohort. “We do not know any aspects of dietary intake in adulthood,” Szilagyi says. “Nor do we know that the adults who developed colorectal cancer were also the very children in the families that indeed had higher dairy intakes.” In light of the fact that most studies have reported a protective effect of dairy products, it is important to determine the extent to which the former diet was continued into adulthood, he notes.

The milk consumption levels identified as posing a significant cancer risk in the Boyd Orr cohort are similar to current average intakes for U.S. children. Nonetheless, the researchers assert that it would be premature to consider altering current guidelines for children’s nutrition. “Dairy products are important contributors to children’s intake of protein, vitamins, and minerals,” says van der Pols. “Because this is only the first study to show associations between childhood dairy consumption and risk of cancer in adulthood, more evidence is needed before any firm conclusions can be drawn.” –M. Nathaniel Mead