Advance in using biopsy samples in understanding environmental causes of cancer

September 9 2013

In an advance in determining the role of environmental agents in causing cancer, scientists today described development of a long-sought way to use biopsy samples from cancer patients to check on human exposure to substances that damage the genetic material DNA in ways that can cause cancer.

Their report on the method, which taps into a treasure trove of medical information in biopsy samples of patients, was part of the 246th National Meeting of the American Chemical Society (ACS).

"This is the first successful use of archived biopsy samples in analyzing exposure and DNA damage, by mass spectrometric methods, of cancer patients to an environmental carcinogen," said Robert J. Turesky, Ph.D., who led the research. "It clears the way for use of this huge resource of archived biopsy samples for gaining insights into cancer and other diseases."

Byeong Hwa Yun, Ph.D., who collaborates with Turesky, gave the presentation. They conducted the study in collaboration with Arthur P. Grollman, M.D., and his associates at Stony Brook University, N.Y.; and with Dr. Bojan Jelakovi? (University of Zagreb) and Dr. Jovan Nikoli? (Clinical Center, Belgrade), and their associates. Yun explained that the samples came from an archive of what scientists term "formalin-fixed paraffin embedded (FFPE) tissues." After doctors take a biopsy, the

sample gets preserved in a solution of formaldehyde called formalin. Pathology lab workers then embed the tissue in a block of wax, and shave off slices as thin as paper so a pathologist can examine it microscopically for cancer or other diseases.

The remaining wax block of tissue typically goes into storage and can be kept indefinitely at room temperature without spoiling. By some estimates, more than 1 billion such FFPE samples are archived around the world, containing a wealth of information that scientists have begun to use in various kinds of research.

Yun explained that scientists who study human exposure to potential cancer-causing substances in the environment long have dreamed of using FFPE samples to look for the tell-tale signs of such exposure. Those biomarkers are "DNA adducts," pieces of DNA where a carcinogenic substance has latched on. The bonding may mean the start of cancer.

"But the only available way of processing those samples to detect DNA adducts had serious drawbacks," said Yun. "It required the use of harsh conditions that caused the DNA to decompose, and the technique had serious drawbacks in identifying the kind of adduct and determining how much was present. Our experimental breakthrough solves those problems and enables broader use of FFPE samples in understanding the role of environmental exposures in cancer risk."

Turesky and Yun, who conducted this research at the New York State Department of Health and are currently with the University of Minnesota, used the approach to detect DNA adducts involving a carcinogen called aristolochic acid in biopsy samples from patients from the Balkans and Taiwan with upper urinary tract cancer. Found naturally in certain plants of the genus *Aristolochia sp.*, aristolochic acid is present in certain traditional Chinese herbal medicines that have been the

subject of health warnings, and the U.S. Food and Drug Administration and regulatory agencies in other countries have banned importation of Aristolochia herbs. Aristolochic acid is also the causal agent of Balkan endemic nephropathy, a devastating environmental disease recently shown to be caused by the ingestion of bread, a major component of the local diet, contaminated with *Aristolochia sp.* Using the new mass spectrometric approach, Turesky and Yun verified the existence of DNA adducts involving aristolochic acid in patients with upper urinary tract cancer, further strengthening the link between this chemical and upper urinary tract cancer.

"With this method, we can retrieve DNA adducts from FFPE samples and, in effect, use them as a dosimeter to measure human exposure to suspected carcinogens," said Yun. "The exposure to chemicals and their DNA adducts may be correlated to mutations in specific genes that can lead to cancer in a patient, and point to specific environmental agents — carcinogens and suspected carcinogens. The methodology will be of major importance in understanding exposures and the role of chemicals in the environment in causing cancer."

Turesky and Yun now are checking FFPE samples for DNA adducts associated with cigarette smoking, cooked meats and air pollution.

More information: Abstract

Human formalin-fixed paraffin-embedded tissues: An untapped specimen for biomonitoring carcinogen DNA adducts by mass spectrometry

DNA adducts are a measure of internal exposure to environmental and endogenous genotoxicants and are useful for risk assessment. Unfortunately, in molecular epidemiologic studies, the measurement of DNA adducts is frequently precluded by the unavailability of fresh

tissue. In contrast, formalin-fixed paraffin embedded (FFPE) tissues are often accessible and represent a rich and largely untapped source for DNA adduct biomarker research. FFPE tissue has not been employed for quantitative measurement of DNA adducts because of the technical challenges in retrieval of high quality DNA that is fully digestible by nucleases. We report here that DNA adducts of aristolochic acids (AAs) can be measured in FFPE tissues at a level of sensitivity comparable to freshly frozen tissue. AAs are nephrotoxic and carcinogenic compounds found in Aristolochia plant species, many of which have been used worldwide for medicinal remedies. AAs are implicated in the etiology of aristolochic acid nephropathy (AAN) and Balkan endemic nephropathy (BEN). 8-Methoxy-6-nitro-phenanthro-[3,4-d]-1,3-dioxolo-5-carboxylic acid (AA-I) is a major component of Aristolochia herbs and a potent human urothelial carcinogen. We established a method to quantitatively retrieve the aristolactam-DNA adduct 7-(deoxyadenosin-N6>-yl) aristolactam I (dA-AL-I) from FFPE tissue. Adducts were measured, using ultra performance liquid chromatography/multistage scan mass spectrometry (UPLC-MS/MS>n>), in liver and kidney tissues of mice exposed to AA-I, at doses ranging from 0.001 to 1 mg/kg body weight. dA-AL-I was then measured in 10 μm thick tissue-sections of FFPE kidney from patients with upper urinary tract cancers. dA-AL-I adduct levels in FFPE were comparable to those levels measured in fresh frozen samples. The limit of quantification of dA-AL-I was 3 adducts per 109 DNA bases per 3 μg of DNA. The ability to retrospectively analyze FFPE tissues for DNA adducts may provide clues to the origin of human cancers for which an environmental cause is suspected.

Provided by American Chemical Society