et al. (CX5CE Synchron Clinical System instrument; Beckman Instruments, Inc., Fullerton, CA) was different from ours. On the other hand, it is noteworthy that we were able to improve the prediction result when we adjusted the Chatelut formula, as suggested by Minami et al. (Fig. 1, B).

We wish to thank Minami et al. for their useful advice and we again should recognize and respect the pioneering work done by Chatelut et al. Furthermore, we again propose that it would be better to include a brief description about the method used for creatinine measurement when giving predicted value of carboplatin area under the curve (AUC) or clearance. Considering the possibility of ethnic differences, as suggested by Chatelut et al. (2), we are now devising both a Japanese-based population pharmacokinetic model and a limited sampling model for carboplatin clearance or AUC prediction (Miyazaki M, Fujiwara Y, Takahashi T, Isobe T, Ohune T, Tsuya T, et al.: manuscript submitted for publication).

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Re: You Say Tomato and I Say Tomahito: Getting a Handle on Pronouncing Apopotosis

Dr. Longo (1) made some relevant points about the pronunciation of apoptosis; however, I would take issue with the statement that “pathologists have been recognizing this process for many years on light microscopic examination, but they called it “karyorrhexis”—“A new word to describe the process may not have been necessary in the first place. . . .” It is not simply karyorrhexis, as Kerr et al. (2) have brilliantly shown. This was a revolution in interpretation of numerous physiologic and pathologic processes, many hitherto thought to be unrelated. This type of cell death occurs in normal as well as pathologic tissues, and of major importance (among many other revelations) was the discovery that the treatment effects of cancer chemotherapy (and other types of chemotherapy) as well as cell death from ionizing radiation came about by apoptosis (3). It is perhaps a little embarrassing for us as microscopists and pathologists to realize we have been observing these now easily recognizable apoptotic cells for many years, not understanding their uniqueness or significance. On the other hand, if it is any consolation, Virchow, Morgagni, or Paget, among many others, also did not appreciate the difference or significance. There are literally hundreds of articles now being published in a variety of scientific journals concerning “apoptosis.” This is a bit more than “karyorrhexis.”

STEPHEN S. STERNBERG

von Hippel-Lindau Disease Gene Alterations Associated With Endolympathic Sac Tumor

Since the first characterization of an endolympathic sac tumor (ELST) in 1984 (1), an increasing number of case reports have described primary low-grade adenocarcinomas of the temporal bone that appear to originate in the endolympathic sac (2). Recently, ELSTs have been identified as a frequent manifestation of von Hippel-Lindau (VHL) disease (3). In a large population of individuals being screened for VHL disease, ELSTs were detected on magnetic resonance imaging (MRI) in 10.7% of the 121 patients with evidence of VHL disease. In contrast, no ELSTs were detected in the 253 individuals without evidence of VHL disease (3). Furthermore, 43 (65%) of 66 unselected patients from the National Institutes of Health VHL clinic had pure tone threshold hearing abnormalities, which occurred bilaterally in 23 of the 43 affected subjects (3). Hearing loss, tinnitus, and vertigo comprise the most common constellation of symptoms in patients with ELSTs.

The tumor-suppressor gene responsible for VHL disease has been mapped to chromosome 3p25 (4) and subsequently identified (5). Recent studies on renal cell carcinomas (6), pheochromocytomas (7), and hemangioblastomas (8) from patients with VHL disease support...
the hypothesis of Knudson (9) that both an inherited germline mutation and loss of function of the wild-type allele of the VHL gene are essential for the development of these neoplasms. To confirm the clinical association of ELSTs with VHL disease, we performed a molecular genetic analysis on an ELST from a patient with genotypically confirmed VHL disease.

A 32-year-old female had been treated previously for retinal angioma, cerebellar hemangioblastoma, and adrenal pheochromocytoma. Several family members had typical features of VHL disease. Genotypic evaluation of the patient disclosed a three-nucleotide germline insertion within exon 2 of the VHL gene. After sudden loss of hearing in the left ear at age 17 years, she had episodic, incapacitating vertigo for 2 years. The vestibular dysfunction abated over several years, but she continued to have profound hearing loss in the left ear with associated constant high-pitched tinnitus.

Audiometric evaluation revealed a profound sensorineural hearing loss in the left ear with an elevated speech awareness threshold of 85 dB and no appreciable speech recognition. Pure tone thresholds and speech scores were normal for the right ear. Acoustic reflexes, evoked responses (auditory brainstem response), and otoacoustic emissions were absent in the left ear, as anticipated from the severity of the hearing loss. MRI and computed tomography (CT) scanning revealed a heterogeneously enhancing 1.8-cm mass in the petrous portion of the temporal bone that was locally destroying bone and extending into the posterior fossa. A small cerebellar hemangioblastoma was also evident. Abdominal CT and MRI scanning revealed a renal tumor, renal cysts, and multiple pancreatic cysts. Because of progressive growth of the tumor on serial MRI over 3 years, surgery was recommended. A vascular tumor with a nodular surface protruding into the posterior fossa from the petrous bone was excised. The tumor had a papillary microscopic architecture with glandular elements lined by single rows of cuboidal cells with eosinophilic or clear cytoplasm.

For genetic analysis, fluorescence in situ hybridization (FISH) and mutation analysis were used. For FISH, a touch preparation was performed from a fragment of frozen tumor. In situ hybridization was performed using a P1 plasmid clone containing the VHL gene as the probe. The DNA was labeled with digoxigenin-11-deoxyuridine triphosphate by nick translation (Boehringer Mannheim Corp., Indianapolis, IN). Slides were denatured in 70% formamide/2× standard saline citrate at 72 °C for 2 minutes and dehydrated in ethanol series of 70%, 80%, 90%, and 100%. The probes were denatured at 70°C for 10 minutes and then incubated at 37°C for preannealing. DNA (250 μg) was applied on the slide and allowed to hybridize overnight in a humidified chamber at 37°C. Detection was performed by use of antidigoxigenin rhodamine. Hybridization signals were scored with the use of a Zeiss Axiohot fluorescence microscope, and three-color images were captured on a Photometrics cooled-CCD camera (Photometrics, LTD, Tucson, AZ) by use of IP Lab image software (Signal Analytics Corporation, Vienna, VA).

For mutation analysis, we extracted DNA from tumor cells that were procured from an unstained 5-μm, formalin-fixed, tissue section by microdissection with the use of a 30-gauge needle as previously described (10). Polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) analyses were performed with primers 55/56, which cover the open/reading frame of exon 2 of the VHL gene. After detection of the mutated allele, DNA was extracted from excised bands of the SSCP gel and amplified with PCR. The amplified PCR products were used for DNA sequencing (Cyclin Sequencing Kit; The Perkin-Elmer Corp., Foster City, CA).

Mutation analysis disclosed a three-nucleotide germline insertion in the VHL gene, and FISH revealed deletion of the opposite allele (Fig. 1). FISH analysis detected deletion of the P1 clone in 80 (80%) of 100 cells examined (Fig. 1, A). Twenty percent showed both alleles, interpreted as stromal cells with a constitutional genotype. By mutation analysis, we found that the intensity of the wild-type allele bands was markedly decreased compared with density of the aberrant electrophoresis bands (Fig. 1, B). Sequencing analysis revealed a three-nucleotide insertion at the splicing site of exon 2 in the aberrant band (Fig. 1, B).

Clinical screening of a large population of individuals at risk for VHL disease has allowed identification of ELSTs as another phenotypic manifestation of VHL disease (3). The results of our genetic analysis of an ELST were from a patient with genotypically

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**Fig. 1.** Genetic analysis of endolymphatic sac tumor (ELST). A) FISH analysis of ELST cells from touch preparation. Only one red signal, which represents the VHL gene in the P1 plasmid clone, is seen in tumor cells; the inset shows stromal cells with two red signals. B) Single-strand conformation polymorphism gel shows aberrant bands in tumor (T) compared with lymphoid control tissue with normal genotype (C). Sequencing analysis reveals a three-nucleotide insertion (GGT) in tumor cells (T) compared with lymphoid control tissue (C).
confirmed VHL disease provide direct genetic evidence that the loss of the VHL gene wild-type allele is associated with tumorigenesis of the endolymphatic sac.

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Inhibition of Mammary Carcinogenesis in Rats by Parenteral High-Dose Vitamin E

The effects of various nutritional antioxidants on carcinogenesis have been studied recently, since lipid peroxidation is thought to be one of the causes of breast cancer. An early study (1) suggested that vitamin E provides protection against mammary cancer. One study (2) reported a decrease in the formation of mammary tumors induced by daunomycin among rats that received an injection of vitamin E. In contrast, several studies have reported that dietary vitamin E is ineffective against 7,12-dimethylbenz(a)anthracene (DMBA)-induced (3) or N-methyl-N-nitrosourea (MNU)-induced (4) mammary cancer. However, it was reported that vitamin E could potentiate the ability of selenium to inhibit the tumorigenesis in DMBA-treated rats (5). Generally, in experimental cancer chemoprevention studies, the doses of vitamin E used to provide protection against the development of cancer are much higher than the nutritional requirement and may be in the toxic range.

In light of this knowledge, we decided to investigate the effect of vitamin E, a strong free-radical scavenger and immune system stimulant at high doses (6), on experimental breast cancer in rats by administering via the intraperitoneal route.

The experiment started when all groups of rats were 50-55 days old. Sprague-Dawley female rats were divided into four groups as follows. In group 1 (control), rats were injected with physiologic saline by the intraperitoneal route three times per week until the experiment was completed. In group 2 (carcinogen-treated group), rats were administered MNU (50 mg/kg body weight) via the jugular vein twice at a 1-month interval (7). In group 3 (vitamin E control group), rats were given vitamin E (75 mg/kg body weight) three times per week intraperitoneally until the experiment was completed. In group 4 (carcinogen plus vitamin E group), rats received an intravenous injection of MNU (50 mg/kg body weight) twice at a 1-month interval and with vitamin E (75 mg/kg body weight) intraperitoneally three times per week until the end of the experiment. Food and water were available ad libitum.

Rats were palpated twice a week to determine the appearance and location of tumors and were killed after the development of tumors. In the course of the experimental period, blood was taken from the tail of animals 1 month after injection of the second MNU dose. Malondialdehyde (MDA) as a lipid peroxidation marker, lipid-bound sialic acid (LBsA) as a tumor marker, and some hematologic parameters were measured. Animals were killed when tumors were noticed by palpation and their blood was collected by cardiac venipuncture. In addition to the biochemical analysis mentioned above, vitamin C levels and ceruloplasmin levels as an acute phase reactant were also measured (Table 1).

All organs, as well as mammary tumors, were examined macroscopically and histologically, and the size, weight, and location of tumors were recorded. In seven of eight MNU-treated rats (87.5%, group 2), mammary papillary carcinoma was observed by histopathologic investigation. Tumors were detected between the 2nd and 5th month, and especially in the 3rd month, after the last MNU injection. In contrast, in all rats treated with MNU plus vitamin E (group 4, 10 rats), no tumorigenesis was observed by macroscopic and histopathologic investigations, but general tissue fibrosis, including breast tissue, was noted in some of them. In addition, some rats had bleeding and inflammation, probably due to the sustained, high-dose vitamin E injections. In a previous report concerning the histopathologic investigation of rat mammary glands, it was observed that histologic properties of adenocarcinoma developed as early as 20 days after administration of intravenous DMBA (8).

In conclusion, our experiments suggest that the intraperitoneal administration of vitamin E might have a protective effect on MNU-induced tumor-