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Esophageal Carcinogenesis in the Rat: A Model for Aerodigestive Tract Cancer

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Abstract A number of chemical carcinogens have been used to study the process of esophageal carcinogenesis. Among the most prominent of these models is the induction of cancer of the esophagus in the rat by the nitrosamine *N*-nitrosomethylbenzylamine (NMBA). In the rat, tumors can occur within 15 weeks of carcinogen administration. The rat model has been used to investigate the mechanism of action of several chemopreventive agents. Among these, the garlic-derived agent diallyl sulfide has been shown to be a specific inhibitor of NMBA metabolism. Other investigators have used the model to seek out the relationship of dietary factors and alcohol in esophageal tumorigenesis. With striking histologic parallels to human esophageal carcinoma, the NMBA model provides useful information to study this cancer. © 1993 Wiley-Liss, Inc.

Key words: esophageal cancer, rat, nitrosomethylbenzylamine, chemoprevention

Cancer of the esophagus is a difficult clinical problem. Because the esophagus is located in the midst of the aerodigestive field, the disease is often accompanied by nutritional dysfunction. Advanced tumors in this location readily seed nearby lymph nodes with local, then metastatic, disease [1].

Cancer of the esophagus is a potential participant in the "field cancerization" hypothesis of aerodigestive tract tumorigenesis [2]. In people with a long history of smoking, successfully removed cancers of the head and neck have a high risk of recurrence, which can occur in the esophagus. As a primary tumor, cancer of the esophagus occurs relatively frequently throughout the world. A number of etiologic agents have been implicated in its development [3].

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ESTABLISHMENT OF A RAT ESOPHAGEAL CANCER MODEL

The spontaneous occurrence of esophageal cancer in rodents is virtually unknown. Capitalizing on the squamous origin of the esophageal epithelium, early efforts were made to induce tumorigenesis in the rat, since it was known as early as the 1930s that epidermal tumors were readily induced by chemically carcinogens [4]. In the last two decades, however, the chemical synthesis of several alkylnitrosamines led to the discovery of organotropism for the esophagus when tested in rodents [5–7]. Among the most potent of the substituted nitrosamines is the compound *N*-nitrosomethylbenzylamine (NMBA) [6,11]. This carcinogen is notable amongst the nitrosamines, a chemical group containing some of the most potent of experimental carcinogens known to man. Due to an aromatic side chain, NMBA is much more lipid-soluble than water-soluble. Early attempts at testing NMBA for carcinogenicity revealed it to

be highly specific for the esophagus in mice and rats [6]. Lesions induced by NMBA in the rat esophagus are histologically similar to human cancers of squamous cell origin. Carcinogenesis in the rat esophagus develops through several intermediate stages. Parenteral injections of NMBA illicit severe esophagitis in the acute stages. From this origin, hyperplastic lesions progress through a papillary stage to squamous cell carcinoma [10,11]. Like many alkylating carcinogens, NMBA is a powerful methylating agent, and the O^6 position of guanine in DNA is a prime target for mutagenesis [8,12]. NMBA-induced rat tumors also show a prevalence for mutations in the *ras* gene, in keeping with many experimentally induced tumors [12]. Much is known about the mechanism of action of NMBA carcinogenesis; the metabolism of this carcinogen has been well characterized [13–16]. Although the final transformation of NMBA may occur in esophageal mucosal cells, it is first metabolized in the liver by cytochrome P-4502E1. This P-450 isozyme catalyzes the oxidation of a number of xenobiotics, including alcohol [17]. Since alcohol use has been epidemiologically linked to increased risk for esophageal cancer, the NMBA model has been used to explore possible mechanisms of carcinogenesis [17–21]. Several dietary factors have also been examined for possible roles as promoters of esophageal cancer. A consistent effect has been shown when rats consumed diets deficient in zinc or retinoids [20–22]. These deficiencies may provoke susceptibility to NMBA carcinogenicity in the esophagus. As in the mouse skin tumor model, the esophagus, once initiated by a chemical carcinogen, is responsive to classical tumor promoters such as phorbol esters [23,24]. The understanding of how NMBA is activated by microsomal enzymes has resulted in several pharmacological attempts to inhibit esophageal carcinogenesis in the rat. It is this feature of the model that has proven very useful for studying the mechanism of action of cancer chemopreventive agents.

CHEMOPREVENTION OF ESOPHAGEAL CANCER

Early studies of esophageal cancer inhibition in the rat focused on the known metabolic fate of NMBA. NMBA is processed through the cytochrome system in the liver to proximate carcinogens thought to require final conversion in the microsomal fraction of esophageal mucosal cells [11,25]. Investigations in our laboratory and in others have identified a naturally occurring sulfur compound in garlic that is highly inhibitory toward NMBA-induced esophageal cancer. This agent, diallyl sulfide, is one of several such chemicals in garlic which account for the flavor and fragrance associated with the herb [26–28]. Diallyl sulfide specifically inhibits cytochrome P-4502E1, modulating the ability of this isozyme to bioactivate NMBA [29,30]. We have observed dose-related inhibition of NMBA carcinogenesis by diallyl sulfide, with complete oblation of esophageal carcinogenesis when 200 mg/kg of this sulfur compound was administered orally to rats prior to exposure to the carcinogen, but not after [9,31]. However, competitive inhibition of P-4502E1 binding may not be the sole function of diallyl sulfide's chemopreventive action. We, and others, have also shown that garlic-derived organosulfur compounds are stimulants of glutathione-*S*-transferase activity, an enzyme complex that functions to detoxify xenobiotics [27,28]. The induction of glutathione-*S*-transferase by diallyl sulfide was measured in hepatic or glandular epithelia; it remains to be determined if induction is measurable in the squamous epithelia of the esophagus. Another naturally occurring compound that inhibits NMBA-induced esophageal cancer is phenylethyl isothiocyanate, a derivative of cruciferous vegetables [32]. This compound is of mechanistic interest in that it bears structural similarity to NMBA.

Certain avenues of chemoprevention research have focused on the potential inhibitory properties of vitamins and minerals in the rat esophageal cancer model. Selenium salts fed to rats and vitamin E tested in mice suppressed NMBA-induced tumorigenesis [33–36]. Our own investigation found, in contrast, that retinyl acetate accelerated tumor formation in the esophagus when given subsequent to the carcinogen [37]. Among the more recent approaches to interventions in the NMBA model are efforts to discover whether factors from diet or drinking habits observed in man impact on the rat model. Promising inhibitory activity has been shown repeatedly when rats are treated with

green tea infusions, or fed tablets of protease inhibitors derived from soybean [38–40].

CONCLUSIONS

Cancer of the esophagus shares many etiologic origins with other tumors of the aerodigestive tract. A rat model has been established that parallels the development of human squamous cell carcinoma of the esophagus. The model has excellent utility for studying the biology of intermediate endpoints such as premalignant lesions. Chemoprevention studies in this model have been useful in dissecting the mechanism of action of selected agents. These studies may be rather limited in their public health relevance, since they are directed to the metabolism of this model carcinogen. Yet the rat model can be employed to investigate other chemopreventive approaches with greater relevance to man, as suggested by recent studies on protease inhibitors and green tea polyphenolics.

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