

FAST TRACK

Prevention of upper aerodigestive tract cancer in zinc-deficient rodents: Inefficacy of genetic or pharmacological disruption of COX-2

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Zinc deficiency in humans is associated with an increased risk of upper aerodigestive tract (UADT) cancer. In rodents, zinc deficiency predisposes to carcinogenesis by causing proliferation and alterations in gene expression. We examined whether in zinc-deficient rodents, targeted disruption of the cyclooxygenase (COX)-2 pathway by the COX-2 selective inhibitor celecoxib or by genetic deletion prevent UADT carcinogenesis. Tongue cancer prevention studies were conducted in zinc-deficient rats previously exposed to a tongue carcinogen by celecoxib treatment with or without zinc replenishment, or by zinc replenishment alone. The ability of genetic COX-2 deletion to protect against chemically-induced forestomach tumorigenesis was examined in mice on zinc-deficient versus zinc-sufficient diet. The expression of 3 predictive biomarkers COX-2, nuclear factor (NF)- κ B p65 and leukotriene A₄ hydrolase (LTA₄H) was examined by immunohistochemistry. In zinc-deficient rats, celecoxib without zinc replenishment reduced lingual tumor multiplicity but not progression to malignancy. Celecoxib with zinc replenishment or zinc replenishment alone significantly lowered lingual squamous cell carcinoma incidence, as well as tumor multiplicity. Celecoxib alone reduced overexpression of the 3 biomarkers in tumors slightly, compared with intervention with zinc replenishment. Instead of being protected, zinc-deficient COX-2 null mice developed significantly greater tumor multiplicity and forestomach carcinoma incidence than wild-type controls. Additionally, zinc-deficient COX-2^{-/-} forestomachs displayed strong LTA₄H immunostaining, indicating activation of an alternative pathway under zinc deficiency when the COX-2 pathway is blocked. Thus, targeting only the COX-2 pathway in zinc-deficient animals did not prevent UADT carcinogenesis. Our data suggest zinc supplementation should be more thoroughly explored in human prevention clinical trials for UADT cancer.

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Upper aerodigestive tract (UADT) cancer, including esophageal and oral cancer, is an important cause of morbidity and mortality worldwide.¹ With a 5-year survival of ~10%, esophageal cancers are deadly. The prognosis of oral cancer, the major site being the tongue, is equally dismal, with an increasing incidence worldwide, particularly in young adults.² Patients with oral cancer have a high mortality rate, because of field cancerization effects that result in second primary tumors, particularly in the esophagus.^{3,4} Thus, new chemopreventive and therapeutic approaches are much needed to prevent and treat these deadly cancers.

While chronic alcohol consumption and tobacco use are the major risk factors for UADT cancer, epidemiologic and clinical studies have implicated dietary zinc deficiency (ZD) in the etiology of esophageal squamous cell carcinoma (SCC) and head and neck SCC.^{5–9} In 2005, Abnet *et al.*¹⁰ provided the strongest evidence of an association between ZD and esophageal cancer in humans, by establishing an inverse relationship between zinc concentration in biopsy samples from a high esophageal SCC incidence area and subsequent risk of developing cancer.

We have developed *in vivo* cancer models that reproduce features of human UADT cancer. In rodents, ZD creates a precancerous condition in the UADT, including esophagus, forestomach (considered a dilation of the lower esophagus) and tongue by causing unrestrained cell proliferation^{11–13} and extensive changes in gene expression, including upregulated expression of genes that are relevant in UADT cancer *cyclooxygenase-2* (COX-2), *MT-1* and *cytokeratin 14* (KRT14).^{14–19} Rats on a ZD diet are exquisitely sensitive to *N*-nitrosomethylbenzylamine (NMBA)-induced esophageal^{20,21} and 4-nitroquinoline 1-oxide (NQO)-induced lingual carcinogenesis.¹³ Zinc replenishment (ZR) rapidly reverses cell proliferation, stimulates apoptosis, corrects abnormal gene expression and inhibits esophageal tumorigenesis.^{13,22} Additionally, *p53*-deficient mice or *cyclin D1* overexpressing transgenic mice on a zinc-deficient diet showed rapid development and progression of esophageal/forestomach tumors by NMBA,^{16,23} as well as lingual/esophageal tumors by NQO.²⁴ Since UADT cancer patients are often zinc-deficient, our well-characterized ZD rodent cancer models offer opportunities to explore the biologic role of zinc in UADT cancer development and prevention.

Targeted molecular intervention and therapies have been explored in attempts to prevent or cure cancer. The rationale for targeting the COX-2 pathway for cancer prevention is supported by numerous preclinical and human studies, culminating in use of celecoxib, a selective COX-2 inhibitor with Food and Drug Administration approval for cancer prevention in patients with familial adenomatous polyposis.²⁵ COX-2 selective inhibitors are actively being tested in clinical trials for the prevention of several cancers, including colorectal, esophageal adenocarcinoma and head and neck SCC.^{26–28}

COX-2, which catalyzes the formation of prostaglandins from arachidonic acid, is induced quickly by factors implicated in carcinogenesis, including growth factors, inflammatory stimuli, oncogenes and tumor promoters.²⁹ The finding that deletion of the COX-2 gene in *Apc* knockout mice greatly reduces intestinal polyp formation provides genetic evidence that COX-2 plays a key role

Abbreviations: (COX-2, cyclooxygenase-2; cxb, celecoxib; KRT14, cytokeratin; LTA₄H, leukotriene A₄ hydrolase; NF- κ B, nuclear factor- κ B; NMBA, *N*-nitrosomethylbenzylamine; NQO, 4-nitroquinoline 1-oxide; PCNA, proliferating cell nuclear antigen; SCC, squamous cell carcinoma; UADT, upper aerodigestive tract cancer; ZD, zinc deficiency; ZR, zinc replenishment; ZS, zinc sufficiency; 5-LOX, 5-lipoxygenase).

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