**ABSTRACT**

Dietary zinc (Zn) deficiency (ZD) in rats induces an inflammatory gene signature that fuels esophageal squamous cell cancer (ESCC). Using nanoString™ technology, we show that the inflammation is accompanied by altered expression of specific microRNAs in esophagus, as well as skin, lung, pancreas, liver, prostate, and PBMC, predictive of disease development. Particularly, the ZD esophagus has a microRNA signature resembling human ESCC tumors, miRNAs with overexpression of miR-31 and miR-21, and downregulation of their respective tumor suppressor targets PPP3R2A and PDCD4. Esophageal miR-31 and miR-21 levels are directly associated with the appearance of ESCC. In situ hybridization localizes miR-31 to tumor and miR-21 to stromal cells, establishing their cell-type specificity. In esophageal tissue sections, ZD shows strong expression of miR-31 in stromal cells, stromal-epithelial junctions, and esophageal cancer, while miR-21 is present in epithelial cells and miR-21 is in PBMC. These data establish that overexpressing miRNAs is a hallmark of human ESCC.

**METHODS**

**Study design:** Male weanling Sprague-Dawley rats were fed an egg white-based ZD or ZS diet for 23 weeks. Blood, esophagus, tongue, skin, lung, liver, prostate, and pancreas were collected. Esophagus and tongue were cut into two parts, one formalin-fixed and paraffin-embedded (FFPE) and the other snap-frozen and stored at -80°C.

**Gene expression profiling:** The nanoString nCounter system (nanoString Technologies) that directly measures miRNA expression levels without enzymatic reactions or bias was used. Total RNA (100 ng) was the input material. Small RNA samples were prepared by ligating a specific DNA tag onto the 3′ end of each mature miRNA. The tags provided identification for each miRNA species in the sample. Following hybridization with a panel of pre-designed probes and U87 (normalizer) were from nanoString, each sample was normalized to the geometric mean of 50 most highly expressed miRNAs. Statistical high-density scan was performed. Each sample was normalized to the expression of the U87 mean.

**Gene ontology (GO) analysis:** Significances of pair-wise comparisons were calculated by student’s t-test. Here we investigated whether overexpression of cancer-related inflammation cells (PBMCs) after prolonged ZD, using the nanoString nCounter technology.

**Immunoblot Analysis:** We investigated if Zn-supplementation that suppresses tongue cancer development and inflammation, downregulates miR-31 and miR-21 expression. To investigate ZD effect, miR-31 and miR-21 expression was compared in tumor and normal tissue, respectively. miR-21 target S100A8/A9, miR-31 target S100A8/A9, respectively, and Zn concentration in ESCC, control and PBMCs.

**RESULTS**

**RESULTS**

**CONCLUSIONS**

This study shows that prolonged dietary ZD induces aberrant microRNA expression in a wide variety of tissues associated with inflammation, suggesting a likely mechanism contributing to the burden of human diseases associated with ZD. Importantly, the demonstration of the dysregulation of miR-31 and miR-21 by dietary ZD in inflammatory esophageal/neoplasmic lesions provides new insight into the mechanisms whereby ZD promotes human ESCC and tongue SCC.

**REFERENCES**


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**REFERENCES**


