Alcohol Induces Reactive Oxygen Species and Migration in Keratinocytes

Alex W. Helkin, BA; Hoang-Lan T. Nguyen, PhD; Ghassan J. Samara, MD

SUNY at Stony Brook, Department of Otolaryngology

ABSTRACT

Ethanol is synergistic with tobacco in HNSCC carcinogenesis. While ethanol or tobacco, alone, increases the risk of HNSCC 2- to 6-fold, this risk increases to 50-fold with both ethanol and tobacco consumption. Reactive oxygen species (ROS) has been implicated in numerous cellular processes associated with malignancy, including enhancement of cell proliferation, DNA damage, and cell migration. The data collected illuminate the previous theories of "field cancerization", which explain the findings of multiple primary tumors in HNSCC. The oral mucosa of smokers is a non-uniform environment in which some cells have a distinctly higher probability of transformation. Increased reactive oxygen species and increased migration happen concurrently, furthering the potential for metastasis of aberrant cells.

INTRODUCTION

Alcohol use has long been thought to be an important risk factor in the development of Head and Neck squamous cell carcinoma (HNSCC), particularly in individuals who concurrently smoke tobacco, where the chance of developing HNSCC increases dramatically. This synergy increases the risk of developing HNSCC from 2- to 6-fold [1, 2] with smoking or alcohol alone, to almost 50-fold when both are concurrently used [3]. Alcohol metabolism generates reactive oxygen species (ROS) within cells, much like those produced in the mitochondria during oxidative phosphorylation. These molecules have been implicated in a variety of cellular mechanisms such as DNA oxidation transforming guanine bases to 8-OHdG, a commonly measured marker of DNA damage, and intracellular signaling, playing roles in the induction of programmed cell death, decreasing contact-inhibition of cell growth [4] and stimulating invasion [5]. The focus of our study is to examine the effects of alcohol on normal keratinocytes and transformed keratinocytes that serve as a model for cells predisposed to malignant mutations (i.e. exposed to tobacco smoke). The oral mucosa of a smoker is a heterogeneous population of cells, with varying potential for epithelial-mesenchymal transformation (EMT) and transition to carcinoma. The aim will be to investigate whether alcohol’s ability to induce ROS plays a role in selecting for cells already mutated by tobacco’s effects, demonstrating the commonly observed phenomenon of “field cancerization” seen particularly in HNSCC [6].

HYPOTHESIS

-We hypothesize that ROS induced by repeated exposure to ethanol, enhances the potential of previously damaged, pre-malignant cells to lose contact inhibition and invade the oral mucosa. ROS is selectively increased in transformed cells, predisposing them to malignant change over normal ones.

RESULTS

-Transformed HaCaT-II-4 cells had a higher basal level of reactive oxygen species than normal HaCaT cells. (Figs. 1, 2)
-When compared to normal HaCaT cells, the transformed HaCaT-II-4 cells showed a 50.7% greater ROS level. (mean fluorescence: HaCaT-II-4 = 6.95; HaCaT = 4.61). (Fig. 3)
-HaCaT-II-4 cells exhibited increased migration in response to ethanol, when compared to HaCaT cells. (Fig. 4)
-The migration response of the HaCaT-II-4 cells was found to be dose-dependent. (Fig. 5)

MATERIALS and METHODS

-Transformed HaCaT-II-4 cells had a greater basal level of ROS than the normal HaCaT cells. While both cell lines showed significant increase in ROS when exposed to 8% ethanol. When compared to normal HaCaT cells, the transformed HaCaT-II-4 showed a 50.7% greater ROS level. (mean fluorescence: HaCaT-II-4 = 6.95; HaCaT = 4.61). Exposure to 8% ethanol significantly increased the migration of transformed HaCaT-II-4 cells, while having little effect on normal HaCaT cell migration.

CONCLUSIONS: The oral mucosa of smokers contains a mixture of normal and transformed keratinocytes, as a result of the mutagenic effects of tobacco. Our results suggest that alcohol preferentially enhances an invasive phenotype in transformed keratinocytes over normal keratinocytes by inducing ROS.

DISCUSSION

-Migration of pre-malignant cells is the first step of invasion. ROS is selectively increased in transformed cells, predisposing them to malignant change over normal ones.

CONTACT

Alex Helkin
SUNY at Stony Brook
Email: alex.helkin@stonybrook.edu
Phone: 631.455.6942

REFERENCES