Anti-cancer effect of hydrogen sulfide: an example of host-bacterial mutualism in oral cancer

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The incidence of oral cancer is lower than that of other common cancers. Understanding the factors that contribute to the decreased incidence of oral cancer may be useful for decreasing the incidence of cancer overall. We focused on the effects of hydrogen sulfide, which is produced by oral bacteria. We report that hydrogen sulfide induced apoptosis in gingival cancer cells that overexpressed pleckstrin homology-like domain, family A, member 1 (PHLDA1), but not in keratinocytes that were derived from healthy gingiva. The anti-cancer effect of hydrogen sulfide may indicate the existence of a host-bacterial mutualism in oral cancer. The role of PHLDA1 as an apoptosis suppressor is also discussed.

Keywords: Hydrogen sulfide; Oral cancer; Oral bacteria; Apoptosis susceptibility; PHLDA1


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Introduction

Elucidation of pathogenesis is the key to prevent all types of disease. In addition, determining ways to stay healthy is useful for lifestyle-related disease prevention. For example, to determine the factors associated with the longevity of Japanese individuals and the low rate of heart disease in French individuals (the so-called “French paradox”), researchers have examined Japanese traditional foods and the polyphenolic compounds present in red wine, respectively. Elucidating the factors associated with the lower incidence of oral cancer may provide clues to decrease the incidence of other types of cancer. We focused on hydrogen sulfide (H\textsubscript{2}S), a common metabolite of anaerobic oral bacteria, as a factor associated with the low incidence of oral cancer.

Cancer of the oral cavity (including the lip) is estimated to be the sixteenth most common cancer worldwide \cite{1}. One of the most common risk factors for carcinogenesis is tobacco smoking. Although the oral cavity is directly exposed to mainstream cigarette smoke, the age-standardized incidence rate of oral cancer is relatively low among tobacco-related cancers \cite{1}.

In mammalian cells, H\textsubscript{2}S is generated from cysteine in reactions catalyzed by cystathionine beta-synthase and cystathionine gamma-lyase \cite{2}. H\textsubscript{2}S plays important roles in numerous aspects of normal physiology and in disease \cite{3,4}; therefore, H\textsubscript{2}S has been recognized as a gaseous signaling molecule, along with nitric oxide and carbon monoxide \cite{5}. A significant amount of H\textsubscript{2}S is produced in various tissues. For example, the endogenous concentration of H\textsubscript{2}S in brain...
tissue has been reported to be 50-160 μM [6]. In the oral cavity, H2S produced by commensal sulfate-reducing bacteria, which are often present in the normal oral microbiota, is the main cause of halitosis [7, 8]. The concentration of H2S in gingival crevicular fluids can be as high as 1.9 mM [9]. It can be anticipated that H2S affects the condition of oral tissues. Persistent H2S exposure induces apoptosis via caspase 9 activity in the gingival cancer cell line Ca9-22, which shows high expression levels of pleckstrin homology-like domain, family A, member 1 (PHLDA1) [10-12]. In contrast, susceptibility to H2S-induced apoptosis was much lower in oral keratinocytes derived from healthy gingiva than in Ca9-22 cells [12]. H2S thus appears to have anti-cancer effects, although the precise mechanisms involved remain unclear.

Epidemiological studies have indicated a relationship between garlic consumption and reduction of the risk of several types of cancer [13], as well as cardiovascular disorders [14]. It has been demonstrated that human red blood cells convert garlic-derived organic polysulfides into H2S, an endogenous cardioprotective vascular cell-signaling molecule [15]. The mechanisms by which garlic consumption prevents cardiovascular diseases are likely due to H2S production [13]. Thus, we proposed that H2S produced from garlic also contributes to induction of apoptosis in mutated cells to explain the observed reduction of cancer risk with garlic consumption.

Expression of PHLDA1 was much higher in Ca9-22 cells than in keratinocytes, whereas expression of PHLDA1 in cells of the lung cancer cell line A549 was low [12] (Fig. 1). Thus, strong expression of PHLDA1 is not specific to cancer cells. PHLDA1 expression was first associated with the restoration of activation-induced apoptosis by coupling T-cell receptor stimulation to Fas expression in T-cell hybridomas [16]. Subsequently, several studies suggested that PHLDA1 has a pro-apoptotic function because PHLDA1 expression was increased during apoptosis [14-21]. PHLDA1 expression also increased during H2S- and actinomycin D-induced apoptosis in Ca9-22 cells [12]. In PHLDA1 knockout mice, however, PHLDA1 was found not to be essential for Fas regulation and apoptosis in vivo [22]. Moreover, no upregulation of PHLDA1 was observed in actinomycin D-induced apoptosis in normal keratinocytes or PHLDA1-negative A549 cells, which indicates that PHLDA1 is not essential for apoptosis [12]. To examine the role of PHLDA1 in apoptosis, we performed knockdown of PHLDA1 expression using small interfering RNA [12]. If the role of PHLDA1 is pro-apoptotic, knockdown should inhibit drug-induced apoptosis or delay the apoptotic response. However, knockdown alone led to induction of apoptosis in Ca9-22 cells [12]. These findings indicated that PHLDA1 act as an apoptosis suppressor. Apoptosis was not induced by PHLDA1 knockdown in keratinocytes [12].

Figure 2 illustrates our hypothesis for the mechanism underlying this differential susceptibility to apoptosis. When cells suffer DNA damage, they attempt to repair the damaged DNA. Irreparable DNA damage in cells will induce apoptosis via the cell’s own apoptosis mechanism, i.e. such cells have a physiological tendency for apoptosis. However, PHLDA1 may block the apoptotic process, resulting in development of PHLDA1-overexpressing cancer cells. In fact, the finding that knockdown of PHLDA1 alone led to induction of apoptosis in PHLDA1-overexpressing Ca9-22 cells indicates PHLDA1-overexpressing cancer cells have a physiological tendency for apoptosis, and that the tendency to apoptosis is blocked by PHLDA1. This tendency for apoptosis may also affect cells’ susceptibility to apoptosis. H2S, which is a comparatively weak apoptotic inducer, may facilitate the physiological tendency for apoptosis to overcome PHLDA1 suppression of apoptosis suppression. The upregulation of PHLDA1 during the process of apoptosis is likely the result of enhanced suppression of apoptosis. In
keratinocytes, apoptosis was not induced by PHLDA1 knockdown because the cells initially lacked an apoptotic tendency. Thus, H$_2$S exposure alone may not be sufficient to induce apoptosis.

It has been reported that PHLDA1-positive cancer cells are more susceptible to anticancer drug-induced apoptosis than PHLDA1-negative cancer cells [12, 20], and that loss of PHLDA1 expression is consistently associated with progression of cancer, which suggests that increased expression of PHLDA1 may characterize a less-aggressive phenotype [20, 23]. The benign phenotype observed with PHLDA1 expression may contribute to the higher apoptotic susceptibility of PHLDA1-overexpressing cancer cells.

Although anti-apoptotic PHLDA1 is a potential target for PHLDA1-overexpressing cancers, careful application of PHLDA1 suppression is necessary. PHLDA1 is highly expressed in epithelial stem cells and is considered a putative epithelial stem cell marker [24, 25]. Thus, proliferative epithelial stem cells likely have high apoptotic susceptibility, and PHLDA1 expression in epithelial stem cells may help to prevent apoptosis in these cells. Therefore, suppression of PHLDA1 expression has the potential to affect not only PHLDA1-overexpressing cancer cells but also epithelial stem cells in vivo. In addition, the physiological tendency for apoptosis in PHLDA1-overexpressing follicular [24] and intestinal [25] epithelial stem cells help to explain hair loss and diarrhea, respectively, as side effects of anticancer therapies involving chemotherapy and radiation therapy.

In conclusion, H$_2$S is an apoptosis inducer for PHLDA1-positive cancer cells that are susceptible to apoptosis. However, H$_2$S is insufficient for apoptosis induction in keratinocytes. These findings indicate that H$_2$S, a common metabolite of anaerobic oral bacteria, is an anticancer compound that may contribute to the low incidence of oral cancer. Further investigation may demonstrate the existence of a host-bacterial mutualism in oral cancer.

**Conflict of interest**

The authors declare that they have no conflicts of interest.

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