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Apoptotic Capacity and Risk of Squamous Cell Carcinoma of the Head and Neck

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Abstract

Background—Tobacco smoke and alcohol drinking are the major risk factors for squamous cell carcinoma of the head and neck (SCCHN). Smoking and drinking cause DNA damage leading to apoptosis, and insufficient apoptotic capacity may favor development of cancer because of the dysfunction of removing damaged cells. In the present study, we investigated the association between camptothecin (CPT)-induced apoptotic capacity and risk of SCCHN in a North American population.

Methods—In a case-control study of 708 SCCHN patients and 685 matched cancer-free controls, we measured apoptotic capacity in cultured peripheral blood lymphocytes (PBLs) in response to in vitro exposure to CPT by using the flow cytometry-based method.

Results—We found that the mean level of apoptotic capacity in the cases (45.9±23.3%) was significantly lower than that in the controls (49.0±23.1%) ($P = 0.002$). When we used the median level of apoptotic capacity in the controls as the cutoff value for calculating adjusted odds ratios (ORs), subjects with a reduced apoptotic capacity had an increased risk (adjusted OR = 1.42, 95%

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Conflict of interest statement

None declared.

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confidence interval [CI] = 1.13–1.78, $P = 0.002$), especially for those who were age ≥57 (1.73, 1.25–2.38, 0.0009), men (1.76, 1.36–2.27, < 0.0001) and ever drinkers (1.67, 1.27–2.21, 0.0003), and these variables significantly interacted with apoptotic capacity ($P_{interaction}$ = 0.015, 0.005 and 0.009, respectively). A further fitted prediction model suggested that the inclusion of apoptotic capacity significantly improved in the prediction of SCCHN risk.

Conclusion—Individuals with a reduced CPT-induced apoptotic capacity may be at an increased risk of developing SCCHN, and apoptotic capacity may be a biomarker for susceptibility to SCCHN.

Keywords

biomarker; Apoptosis; CPT, apoptotic capacity; head and neck cancer

1. Introduction

Head and neck cancer is a heterogeneous group of tumors that involve multiple sites and cellular origins within the head and neck region. Squamous cell carcinoma of the head and neck (SCCHN), including cancers of the oral cavity, oropharynx, hypopharynx, and larynx, is the most common histological type (i.e., 90% of cases) and one of the six most common cancers worldwide [1]. In the United States, approximately 60,000 new cases are diagnosed annually and 12,000 die of this disease each year [2]. It is well known that tobacco smoke and alcohol use as well as prior human papilloma virus (HPV) infection (particularly for the oropharynx subsite) are the major risk factors that play an important role in the etiology of SCCHN. However, only a small fraction of smokers and/or drinkers will develop SCCHN, and a small proportion of those exposed to HPV will develop oropharyngeal cancer (OPC), suggesting that there is genetic susceptibility to this disease in the general population [3–5]. Indeed, tobacco smoke and consumption of alcoholic beverages contain several carcinogens that can cause different types of DNA damage in the target cells [6, 7]. Apoptosis functions to eliminate damaged cells and thus is a critical factor in the protection against tobacco and alcohol induced cancers, including SCCHN [8].

Apoptosis, also called programmed cell death, is a critical mechanism to maintain the balance of cell survival and death by removing irreparable damaged cells [9–11]. Cells have two main apoptosis signaling pathways, an extrinsic (death receptor) and an intrinsic (mitochondrial) pathway [12–14]. The extrinsic pathway, or the death receptor pathway, is initiated from outside of the cell, usually when conditions in the extracellular environment determine cell death. This pathway can be induced through the activation of death receptors, such as death receptor 3 (DR3), death receptor 4 (DR4), death receptor 5 (DR5), FAS and tumor necrosis factor alpha receptor (TNFαR), by their respective ligands. The intrinsic pathway, or the mitochondrial pathway, begins when DNA damage occurs within the cell. It involves a diverse array of non-receptor-mediated stimuli that produce intracellular signals that act directly on targets within the cell. In both pathways, the signaling leads to activation of the caspase family proteases responsible for dismantling and removing the dying cell [15–17]. It is well known that apoptosis contributes to a wide variety of physiological and pathological processes, and dysregulation of apoptosis can disrupt the delicate balance between cell proliferation and cell death, leading to diseases including cancer [9, 18].

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Increasing evidence has demonstrated that insufficient apoptotic capacity can promote development of cancer [19, 20]. Therefore, it is important to detect apoptotic levels and their association with cancer risk through epidemiologic research.

There are many different experimental methods that can induce apoptosis *in vitro*, including radiation, benzo[a]pyrene-7,8–9,10-diol epoxide (BPDE), thapsigargin and camptothecin (CPT), which have somewhat different underlying mechanisms [21–26]. For example, CPT is an effective chemotherapeutic drug for treatment of patients with cancer [27]. Although the mechanisms underlying CPT-mediated responses in cancer cells are not fully understood, it has been identified that CPT induces apoptosis by inhibiting topoisomerase I, resulting in high levels of internally accumulated DNA double-strand breaks in the cell, ultimately leading to cell death [28–30]. CPT is believed to possess promising anticancer effect against a broad spectrum of cancer cell lines, such as those of the breast, colon, lung, and ovarian cancers, because many cancer cells exhibit downregulation of the topoisomerase I activity [30, 31]. To date, most experimental studies of CPT-induced apoptosis were conducted with some cancer cell lines and animal models [22, 25, 27, 32], and there are no reported assays that measure the CPT-induced apoptotic capacity in humans, such as using peripheral blood lymphocytes (PBLs) by flow cytometry in epidemiologic research. In the present study, by measuring the CPT-induced apoptotic capacity in PBLs with a flow cytometry-based Terminal Transferase dUTP Nick End Labeling (TUNEL) assay as previously described [33], we tested the hypothesis that suboptimal apoptotic capacity measured in PBLs is associated with risk of SCCHN in a case-controls study of 703 cases and 685 cancer-free controls of a North American population.

2. Materials and Methods

2.1. Study subjects

The analysis included a total 703 cases who were patients with newly diagnosed, untreated primary tumors of the oral cavity (n = 214; 30.4%), oropharynx (n = 367; 52.4%), hypopharynx (n = 20; 2.8%), larynx (n = 101; 14.4%), and unknown primaries (n = 1; 0.1%). These cases were histologically confirmed and recruited at The University of Texas M.D. Anderson Cancer Center during the period between November 2005 and November 2012. Patients with second SCCHN primary tumors, primary tumors of the nasopharynx or sinonasal tract, or any histopathologic diagnosis other than SCCHN were excluded. By frequency matching on age (±5 years), sex, and ethnicity, we also identified an additional 685 cancer-free control subjects from among hospital visitors at M.D. Anderson Cancer Center during the same time period. Having provided a written informed consent, each of cases and controls completed a self-administered life-style questionnaire that collected information about risk factors, such as tobacco smoking and alcohol use, and donated a one-time sample of 30 ml of blood for biomarker tests. The University of Texas M.D. Anderson Cancer Center Institutional Review Board approved the research protocol.

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2.2. Human peripheral blood lymphocytes (PBLs) culture and camptothecin (CPT) treatment

The method of PBL culture and CPT treatment have been previously reported [33]. Briefly, the PBLs were isolated from the whole blood by using Ficoll (Pharmacia Biotech Inc., Piscataway, NJ) gradient centrifugation and then cultured in RPMI 1640 supplemented with 15% fetal calf serum (GIBCO BRL) and 56.25 μg/ml phytohemagglutinin (Murex Diagnostics, Norcross, GA) for 48 hours at 37°C in an incubator with 5% CO_2. We used CPT to selectively induce apoptosis of the PBLs. The baseline or spontaneous apoptosis index was obtained from the same individual's samples that were not incubated in parallel with those treated by CPT. The dose of 250 nmol/L CPT (Cat# C9911; Sigma-Aldrich, Inc.) for the *in vitro* treatment of the cells for 24 hours was determined according to a previous report [22]. At the indicated time points, the aliquotted samples of cultured cells were harvested and fixed with 1% paraformaldehyde, washed with 1x PBS and finally stored in 70% ethanol at −20°C until used for the apoptotic detection by a flow cytometer.

2.3. Apoptotic analysis with flow cytometry

For the measurement of CPT-induced apoptotic capacity, we used the TUNEL assay as previously described [33]. Briefly, we detected apoptotic cells with an APO-Brdu kit (Phoenix Flow Systems, San Diego, CA), which contained apoptosis-positive and -negative cells as the assay controls, and we used a two-color staining method for labeling DNA breaks and total cellular DNA. The kit included washing, reaction, and rinsing buffers for processing each step of the assay; terminal deoxynucleotidyl transferase enzyme, bromodeoxyuridine triphosphate, and fluorescein-labeled anti-bromodeoxyuridine antibody for labeling DNA; and propidium iodide/RNase A solution for counterstaining the total DNA. The ratio of apoptotic cells (1×10^5) was calculated based on the measurements by flow cytometry (with the Epics Profile II Flow Cytometer, Beckman Coulter, Inc.) according to the instructions of the manufacturer.

2.4. Statistical analysis

The differences in selected demographic variables, smoking, and alcohol use between SCCHN cases and controls were evaluated by using the χ^2-test. The two-sided Student's *t* test was used to test the difference between cases and controls for baseline and CPT- induced apoptotic capacity levels. The apoptotic capacity was determined by the formula [(Percent experimental apoptosis – percent spontaneous apoptosis)/(100 – percent spontaneous apoptosis)] × 100% [34]. The median apoptotic capacity (%) of control subjects was used as the cutoff value: values greater than or equal to the median were considered high levels of apoptotic capacity, and values below the median were considered low/suboptimal levels of apoptotic capacity. The quartiles of apoptotic capacity in controls subjects were used as the cut-off values to evaluate the apoptotic capacity dose-response effect on SCCHN risk. We also dichotomized apoptotic capacity by the median of controls to evaluate its effect on risk of SCCHN by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from both univariate and multivariate logistic regression models in the case-control analysis. These analyses were performed with or without adjustment for age (in years), sex, smoking and drinking status. Subjects who had smoked <100 cigarettes in their lifetimes were defined

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as never smokers; all others were defined as ever smokers. Among ever smokers, those who had quit and had not smoked for >1 year before the interview were defined as former smokers and the others were defined as current smokers. Similarly, subjects who had reported drinking alcoholic beverages at least once a week and longer than 1 year prior to diagnosis or interview were defined as ever drinkers and the remaining as never drinkers. Among ever smokers, those who had quit drinking for longer than 1 year prior to diagnosis or interview were defined as former drinkers and the others as current drinkers.

We also performed a stratified analysis followed by an assessment of interactions between the quartiled apoptotic capacity and selected variables, such as age, sex, ethnicity, smoking and drinking status, tumor sites and stages as previously described [35, 36]. The multiplicative interaction was estimated by including the interaction term (i.e., the quartiled apoptotic capacity × risk factor) in one multivariate logistic regression model, which also included the main effect of the quartiled apoptotic capacity and other covariates. To assess the effect of apoptotic capacity on SCCHN risk prediction, receiver operating characteristic (ROC) curves were plotted from the logistic regression models, and the area under the curve (AUC) were used to assess the classification performance of the models. Statistical significance of the improvement in AUC after adding apoptotic capacity to the model with existing covariates (e.g., age, sex, ethnicity, smoking and drinking status) was calculated by Delong's test as previously described [36, 37]. All tests were two sided, and all statistical analyses were performed with the SAS software (version 9.1.3; SAS Institute, Inc., Cary, NC).

3. Results

3.1. Characteristics of the study population

As shown in Table 1, there were no significant differences in the distributions of age and sex between 703 cases and 685 cancer-free controls, with a similar age distribution ($P = 0.745$) with mean ages of 56.9 (±10.7) and 56.8 (±11.1) years, respectively, but the cases were more likely than the controls to be ever smokers (64.4% versus 46.9%) or ever drinkers (73.4% vs. 57.0%). Furthermore, the differences in tobacco smoke and alcohol consumption between cases and controls were statistically significant (both $P < 0.001$). However, all these variables were further adjusted for any confounding effect in the multivariate logistic regression models later. Overall disease stage distribution of SCCHN patients was 13.8%, 10.3%, 14.4%, and 61.5% for stages I–IV, respectively (Table 1).

3.2. Association between apoptotic capacity and risk of SCCHN

When apoptotic capacity (%) was analyzed as continuous variable, the mean apoptotic capacity in PBLs was significantly lower in the cases than in the controls (45.9+23.3% vs. 49.0+23.1%; $P < 0.002$; Table 2; Supplementary Figure 1). As shown in Table 2, in the controls, men, ever smokers and drinkers had significantly higher apoptotic capacity than did women, never smokers and drinkers ($P < 0.0001$, 0.003 and <0.0001, respectively); in the cases, the younger subjects (<57 years) had significantly higher apoptotic capacity than did older subjects (≥57 years, $P = 0.025$). There were no other significant differences in apoptotic capacity by subgroups of selected variables for both cases and controls. In the

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case-control analysis, we found that the cases were more likely to have a lower apoptotic capacity than the controls, particularly in the older subjects (≥57 years, $P = 0.0009$), men ($P = <0.0001$), Caucasians ($P = 0.031$), ever smokers ($P = 0.005$), ever drinkers ($P < 0.0001$), and OPC ($P = 0.024$) (Table 2; Supplementary Figure 1). We also controlled for multiple testing with the Bonferroni method, and found the results of older subjects, males and ever drinkers passed the Bonferroni threshold (0.05/38 tests = 0.0013) (Table 2).

When apoptotic capacity values were categorized by the median or quartile of the controls, the decreased apoptotic capacity was consistently associated with an increased risk of SCCHN, as assessed in the logistic regression models. As shown in Table 3, when the dichotomized apoptotic capacity was used, we found that individuals with a low apoptotic capacity had a significantly increased risk (i.e., OR with adjustment for age, sex, ethnicity, smoking and drinking status) of SCCHN (adjusted OR = 1.42; 95% confidence interval [CI] = 1.13–1.78, $P = 0.002$), compared with those with a high apoptotic capacity. Similarly, compared with the highest apoptotic capacity quartile (≥67.9%) of the controls, this increased risk was dose dependent [the second highest quartile (57.3–67.8): adjusted OR, 1.18; 95% CI, 0.85–1.65; the third quartile (31.4%–57.2%): adjusted OR, 1.49; 95% CI, 1.08–2.06; and the fourth quartile (<31.4%): adjusted OR, 1.61; 95% CI, 1.17–2.22, respectively; $P = 0.015$]. This trend of increasing risk with decreasing apoptotic capacity was statistically significant ($P_{trend} = 0.002$; Table 3, Figure 1).

3.3. Stratified analysis of apoptotic capacity by selected variables

We assessed possible interactions on multiplicative scales, between apoptotic capacity and the related variables listed in Table 4. We found a significant interaction of apoptotic capacity with age and sex as well as drinking status ($P_{interaction} = 0.015$, 0.005 and 0.009, respectively) on SCCHN risk, which suggested that older subjects (≥57 years), men and ever drinkers with low apoptotic capacity had the highest risk of SCCHN, as compared with their corresponding baseline group (Figure 2).

We further performed stratified analyses to evaluate the effects of apoptotic capacity on the risk of SCCHN in subgroups of age, sex, ethnicity, smoking status, alcohol consumption, tumor sites and tumor stages using the median value in controls for calculating ORs. As shown in Table 4, the stratified analysis showed that the inverse association of apoptotic capacity with SCCHN risk remained significant among subjects with the following characteristics: cases were more likely than the controls to have reduced apoptotic capacity: 1.73 times for the older subjects (adjusted OR = 1.73; 95% CI = 1.25–2.38, $P = 0.0009$); 1.76 times for male (adjusted OR = 1.76; 95% CI = 1.36–2.27, $P < 0.0001$); 1.48 times for ever smokers (adjusted OR = 1.48; 95% CI = 1.10–1.99, $P = 0.009$); 1.67 times for ever drinkers (adjusted OR = 1.67; 95% CI = 1.27–2.21, $P = 0.0003$); 1.59 times for OPC (adjusted OR = 1.59; 95% CI = 1.21–2.07, $P = 0.0008$); 1.47 times for T1/T2 stages (adjusted OR = 1.47; 95% CI = 1.15–1.89, $P = 0.002$); 1.49 times for N2/N3 stages (adjusted OR = 1.49; 95% CI = 1.14–1.94, $P = 0.003$); and 1.51 times for stages III/IV (adjusted OR = 1.51; 95% CI = 1.18–1.93, $P = 0.001$).

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3.4. Prediction of the SCCHN risk by apoptotic capacity

To evaluate the performance of apoptotic capacity in SCCHN risk prediction models, we calculated the AUC using multivariate logistic regression and ROC. As shown in Figure 3, the model included age, sex, race, smoking and drinking status as classifiers, it had an AUC of 63.2%; with the addition of apoptotic capacity, the AUC was significantly improved to 64.4% ($P = 0.029$, by the DeLong's test). With age, sex, ethnicity, smoking and drinking status as classifiers, the model specific for OPC had an AUC of 63.9%; with the addition of apoptotic capacity, the AUC was significantly improved to 66.0% ($P = 0.010$, by the DeLong's test).

4. Discussion

In this hospital-based case-control study, we observed that SCCHN patients had a significantly lower CPT-induced apoptotic capacity in their PBLs than that of the controls. When using the median level of apoptotic capacity in the controls as a cutoff, individuals with a lower apoptotic capacity had a 1.42 times greater risk for having SCCHN than those with a higher apoptotic capacity, which suggests that reduced apoptotic capacity may play a role in the etiology of SCCHN. Stratified analysis further showed that this association was more evident in subgroups of older subjects (≥57 years), men, ever smokers, ever drinkers, OPC, tumor stages T1/T2, N2/N3, and overall stages III/IV. These findings are consistent with the notion that apoptosis capacity may be involved in cancer risk associated with exposure to tobacco smoke and alcohol drink, because old subjects, men and ever smokers and drinkers are more likely to have a higher level of exposure to tobacco smoke and alcoholic beverage [5, 38]. Our additional analyses of the AUC further suggested that apoptotic capacity could significantly improve the prediction of SCCHN risk.

Apoptosis plays a crucial role in many biological and cellular processes, while dysfunction of apoptosis may lead to oncogenesis and development of cancer [18, 39]; thus, evasion of apoptosis is considered one of the hallmarks of human cancers [40]. In fact, pro-apoptotic genes might act as tumor suppressors, whereas oncogenes might fulfill anti-apoptotic functions [40]. Apoptosis defects are now considered an important complement of proto-oncogene activation [41], and establishing targeted pro-apoptotic therapeutic protocols and developing apoptosis-inducing drugs have been under way since the 1990s, which have provided novel approaches to the successful treatment of cancers [42].

Apoptotic capacity has been suggested as a biomarker for risk of several types of cancer, including SCCHN, but results from epidemiologic studies have been inconclusive [19, 21, 43–45]. For example, Zhao *et al.* found that among 30 lung cancer patients and 22 controls, apoptosis levels were significantly lower in the cases than in controls [43]. In a pilot case-control study of 29 patients with neoplasms of the salivary and thyroid glands and 29 cancer-free control subjects, we observed that the low apoptotic capacity was associated with a 10 or four-fold increased risk of salivary and thyroid carcinoma [46]. In a study of 68 lung cancer patients and 74 cancer-free controls, we measured the apoptotic capacity in their cultured PBLs in response to *in vitro* exposure to an ultimate tobacco carcinogen, benzo[a]pyrene diol epoxide (BPDE), by using the terminal dUTP nucleotide end labeling (TUNEL) and flow cytometry, we observed a significantly lower apoptotic capacity in the

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cases than in the controls [19]. Similarly, in a 243 female breast cancer patients and 75 cancer-free controls after their PBLs were irradiated or mock-treated, breast cancer patients shown significantly reduced apoptotic response, compared with normal controls [44]. While in a study of 211 breast cancer patients and 170 cancer-free controls, Docherty *et al.* investigated the apoptotic response to ionizing radiation in PBLs, but the authors failed to reveal any difference in DNA damage-induced apoptosis between the cases and the controls [47].

In the present study that included 703 SCCHN cases and 685 matched cancer-free controls, we found an association between apoptotic capacity and risk of SCCHN, a finding consistent with previous studies that found that a low apoptotic capacity in PBLs was associated with an increased risk of several types of cancers, such as lung cancer [19, 43], breast cancer [44], and salivary and thyroid carcinoma [46]. Our finding also supports a previous study in which the Bcl-2 protein, an inhibitor of apoptosis, played a role in the regulation of T lymphocyte apoptosis [48]. In one relatively small study of 23 laryngeal carcinoma cases and 20 healthy controls in a Poland population, the expression of the Bcl-2 protein in T lymphocytes in $CD4^+$ and $CD8^+$ T lymphocytes measured by flow cytometry was significantly higher in laryngeal carcinoma patients than in the controls [48]. More recently, in another study of 43 SCCHN cases' tumor tissues and 10 normal mucosa samples in a Chinese population, a higher expression of survivin (also called BIRC5, an inhibitor of apoptosis-related protein) in the nuclei of tumor cells than in those of normal mucosa cells [49], and one study identified that survivin inhibited apoptosis through inhibiting both Bax and Fas-induced apoptotic pathways [50]. In contrast with our observations, another study assessed the apoptosis levels using the TUNEL assay by flow cytometry in peripheral blood mononuclear cells from subjects of 22 head and neck cancer patients after treatment by surgery, or surgery and radiation or surgery and chemoradiotherapy and 16 healthy controls and found that cancer patients showed higher apoptotic response than healthy subjects at the time of blood draw (0 time) and after 24 hours incubation [51]. Such a small study is likely to generate unstable estimates.

In the stratified analysis, we also observed that OPC patients had a significantly lower apoptotic capacity in PBLs than that of the controls. It is well known that HPV infection has been implicated as the most important risk for the development of OPC. Among the known HPV types, high-risk HPV-16 is the most common, accounting for approximately 90% or more of HPV-positive OPC cases [52]. There is an increasing molecular evidence that HPV causes human cancers by expressing E6 oncogenic protein, which not only binds to p53 and inhibits its activity, resulting in reduced protein function, but also inhibits p53-independent apoptotic pathways, promoted by different stimuli [53–55]. It has been reported that HPV-16 E6 inhibited drug-induced apoptosis in cells lacking p53 [56]. In transgenic mice expressing HPV-16 E6 in the ocular lens, it has been identified that HPV E6 prevented apoptosis in both wild-type and p53-null animals [57]. Our finding in the present study provided additional epidemiologic evidence that low apoptotic capacity is associated with an increased risk of HPV-related OPC.

To the best of our knowledge, the present study is the first larger population study to epidemiologically show the association of reduced apoptotic capacity with an increased risk

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of SCCHN. However, the present study also had several limitations. First, this is a hospital-based case-control study of the CPT-induced apoptotic capacity assay with some inherited selection bias, although we had applied a stringent frequency-matching strategy in recruiting cases and controls to control for potentially confounding variables. Second, no information of genetic susceptibility was involved in the present study, although such as apoptotic gene mutations and single nucleotide polymorphisms (SNPs) may also influence apoptotic capacity in individuals [19, 58], but the phenotypic measurement of apoptosis may have reflected the sum of all possible genetic variants. Third, although reduced apoptotic capacity has been found to be associated with increased SCCHN risk, we cannot exclude the influence of other potential confounder (e.g., HPV infection status) that was not controlled in the present study, and the retrospective nature of the present study may not exclude the possibility that the disease status may have some effect on the apoptotic capacity. Fourth, the apoptotic capacity was measured in PBLs that were used as a surrogate for head and neck epithelial cells; however, it has been reported that lymphocytes are a good surrogate for estimating cancer risk associated with apoptosis phenotypes as markers for genetic susceptibility [19, 41]. Finally, variations in apoptotic capacity may be due to difference in the interval between blood sample collection and the cell-based experiments, because no repeated blood samples were collected in the study design.

In conclusion, the present study offers the first evidence that reduced CPT-induced apoptotic capacity in PBLs is associated with an increased risk of SCCHN. If our results are validated in large, prospective cohort studies, this apoptotic phenotype could be used as a biomarker for identifying individuals at risk of SCCHN, who might benefit from targeted cancer prevention programs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AC	apoptotic capacity
CPT	camptothecin
PBLs	peripheral blood lymphocytes
SCCHN	squamous cell carcinoma of the head and neck

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OR	odds ratio
CI	confidence interval

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005; 55(2): 74–108. [PubMed: 15761078]
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015; 65(1):5–29. [PubMed: 25559415]
3. De Petrini M, Ritta M, Schena M, Chiusa L, Campisi P, Giordano C, et al. Head and neck squamous cell carcinoma: role of the human papillomavirus in tumour progression. New Microbiol. 2006; 29(1):25–33. [PubMed: 16608122]
4. Hashibe M, Brennan P, Chuang SC, Boccia S, Castellsague X, Chen C, et al. Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiol Biomarkers Prev. 2009; 18(2):541–50. [PubMed: 19190158]
5. Lee YC, Boffetta P, Sturgis EM, Wei Q, Zhang ZF, Muscat J, et al. Involuntary smoking and head and neck cancer risk: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiol Biomarkers Prev. 2008; 17(8):1974–81. [PubMed: 18708387]
6. Pfeifer GP, Denissenko MF, Olivier M, Tretyakova N, Hecht SS, Hainaut P. Tobacco smoke carcinogens, *DNA damage and p53 mutations in smoking-associated cancers*. Oncogene. 2002; 21(48):7435–51. [PubMed: 12379884]
7. Lachenmeier DW, Przybylski MC, Rehm J. Comparative risk assessment of carcinogens in alcoholic beverages using the margin of exposure approach. Int J Cancer. 2012; 131(6):E995–1003. [PubMed: 22447328]
8. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature. 2001; 411(6835): 366–74. [PubMed: 11357144]
9. Danial NN, Korsmeyer SJ. Cell death: critical control points. Cell. 2004; 116(2):205–19. [PubMed: 14744432]
10. Danial NN. BCL-2 family proteins: critical checkpoints of apoptotic cell death. Clin Cancer Res. 2007; 13(24):7254–63. [PubMed: 18094405]
11. Degterev A, Boyce M, Yuan J. A decade of caspases. Oncogene. 2003; 22(53):8543–67. [PubMed: 14634618]
12. Fesik SW. Promoting apoptosis as a strategy for cancer drug discovery. Nat Rev Cancer. 2005; 5(11):876–85. [PubMed: 16239906]
13. Zeiss CJ. The apoptosis-necrosis continuum: insights from genetically altered mice. Vet Pathol. 2003; 40(5):481–95. [PubMed: 12949404]
14. Andersen MH, Becker JC, Straten P. Regulators of apoptosis: suitable targets for immune therapy of cancer. Nat Rev Drug Discov. 2005; 4(5):399–409. [PubMed: 15864269]
15. Pop C, Salvesen GS. Human caspases: activation, specificity, and regulation. J Biol Chem. 2009; 284(33):21777–81. [PubMed: 19473994]
16. Portt L, Norman G, Clapp C, Greenwood M, Greenwood MT. Anti-apoptosis and cell survival: a review. Biochim Biophys Acta. 2011; 1813(1):238–59. [PubMed: 20969895]
17. Martinou JC, Youle RJ. Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. Dev Cell. 2011; 21(1):92–101. [PubMed: 21763611]
18. Wong RS. Apoptosis in cancer: from pathogenesis to treatment. J Exp Clin Cancer Res. 2011; 30:87. [PubMed: 21943236]
19. Wang LE, Cheng L, Spitz MR, Wei Q. Fas A670G polymorphism, apoptotic capacity in lymphocyte cultures, and risk of lung cancer. Lung Cancer. 2003; 42(1):1–8. [PubMed: 14512182]
20. Hassan M, Watari H, AbuAlmaaty A, Ohba Y, Sakuragi N. Apoptosis and molecular targeting therapy in cancer. Biomed Res Int. 2014; 2014:150845. [PubMed: 25013758]

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21. Barber JB, West CM, Kiltie AE, Roberts SA, Scott D. Detection of individual differences in radiation-induced apoptosis of peripheral blood lymphocytes in normal individuals, ataxia telangiectasia homozygotes and heterozygotes, and breast cancer patients after radiotherapy. Radiat Res. 2000; 153(5 Pt 1):570–8. [PubMed: 10790278]
22. Han Z, Wei W, Dunaway S, Darnowski JW, Calabresi P, Sedivy J, et al. Role of p21 in apoptosis and senescence of human colon cancer cells treated with camptothecin. J Biol Chem. 2002; 277(19):17154–60. [PubMed: 11877436]
23. Silva MF, Khokhar AR, Qureshi MZ, Farooqi AA. Ionizing radiations induce apoptosis in TRAIL resistant cancer cells: in vivo and in vitro analysis. Asian Pac J Cancer Prev. 2014; 15(5):1905–7. [PubMed: 24716909]
24. Hsieh WC, Hsu PC, Liao YF, Young ST, Wang ZW, Lin CL, et al. Overexpression of ornithine decarboxylase suppresses thapsigargin-induced apoptosis. Mol Cells. 2010; 30(4):311–8. [PubMed: 20814750]
25. Tan C, Cai LQ, Wu W, Qiao Y, Imperato-McGinley J, Chen GQ, et al. NSC606985, a novel camptothecin analog, induces apoptosis and growth arrest in prostate tumor cells. Cancer Chemother Pharmacol. 2009; 63(2):303–12. [PubMed: 18373093]
26. Hu Z, Li C, Chen K, Wang LE, Sturgis EM, Spitz MR, et al. Single Nucleotide Polymorphisms in Selected Apoptotic Genes and BPDE-Induced Apoptotic Capacity in Apparently Normal Primary Lymphocytes: A Genotype-Phenotype Correlation Analysis. J Cancer Epidemiol. 2008; 2008:147905. [PubMed: 20445773]
27. Zeng CW, Zhang XJ, Lin KY, Ye H, Feng SY, Zhang H, et al. Camptothecin induces apoptosis in cancer cells via microRNA-125b-mediated mitochondrial pathways. Mol Pharmacol. 2012; 81(4): 578–86. [PubMed: 22252650]
28. Paquet C, Schmitt E, Beauchemin M, Bertrand R. Activation of multidomain and BH3-only pro-apoptotic Bcl-2 family members in p53-defective cells. Apoptosis. 2004; 9(6):815–31. [PubMed: 15505424]
29. Furuta T, Hayward RL, Meng LH, Takemura H, Aune GJ, Bonner WM, et al. p21CDKN1A allows the repair of replication-mediated DNA double-strand breaks induced by topoisomerase I and is inactivated by the checkpoint kinase inhibitor 7-hydroxystaurosporine. Oncogene. 2006; 25(20): 2839–49. [PubMed: 16407843]
30. Gilbert DC, Chalmers AJ, El-Khamisy SF. Topoisomerase I inhibition in colorectal cancer: biomarkers and therapeutic targets. Br J Cancer. 2012; 106(1):18–24. [PubMed: 22108516]
31. Desai SD, Li TK, Rodriguez-Bauman A, Rubin EH, Liu LF. Ubiquitin/26S proteasome-mediated degradation of topoisomerase I as a resistance mechanism to camptothecin in tumor cells. Cancer Res. 2001; 61(15):5926–32. [PubMed: 11479235]
32. Jayasooriya RG, Choi YH, Hyun JW, Kim GY. Camptothecin sensitizes human hepatoma Hep3B cells to TRAIL-mediated apoptosis via ROS-dependent death receptor 5 upregulation with the involvement of MAPKs. Environ Toxicol Pharmacol. 2014; 38(3):959–67. [PubMed: 25461556]
33. Li C, Lu J, Liu Z, Wang LE, Zhao H, El-Naggar AK, et al. The six-nucleotide deletion/insertion variant in the CASP8 promoter region is inversely associated with risk of squamous cell carcinoma of the head and neck. Cancer Prev Res (Phila). 2010; 3(2):246–53. [PubMed: 20086182]
34. Fanzo JC, Hu CM, Jang SY, Pernis AB. Regulation of lymphocyte apoptosis by interferon regulatory factor 4 (IRF-4). J Exp Med. 2003; 197(3):303–14. [PubMed: 12566414]
35. Li C, Zhao H, Hu Z, Liu Z, Wang LE, Gershenwald JE, et al. Genetic variants and haplotypes of the caspase-8 and caspase-10 genes contribute to susceptibility to cutaneous melanoma. Hum Mutat. 2008; 29(12):1443–51. [PubMed: 18563783]
36. Wang LE, Hu Z, Sturgis EM, Spitz MR, Strom SS, Amos CI, et al. Reduced DNA repair capacity for removing tobacco carcinogen-induced DNA adducts contributes to risk of head and neck cancer but not tumor characteristics. Clin Cancer Res. 2010; 16(2):764–74. [PubMed: 20068090]
37. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988; 44(3):837–45. [PubMed: 3203132]

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38. Purdue MP, Hashibe M, Berthiller J, La Vecchia C, Dal Maso L, Herrero R, et al. Type of alcoholic beverage and risk of head and neck cancer–a pooled analysis within the INHANCE Consortium. Am J Epidemiol. 2009; 169(2):132–42. [PubMed: 19064644]
39. Cotter TG. Apoptosis and cancer: the genesis of a research field. Nat Rev Cancer. 2009; 9(7):501–7. [PubMed: 19550425]
40. Olsson M, Zhivotovsky B. Caspases and cancer. Cell Death Differ. 2011; 18(9):1441–9. [PubMed: 21455218]
41. Green DR, Evan GI. A matter of life and death. Cancer Cell. 2002; 1(1):19–30. [PubMed: 12086884]
42. Jia LT, Chen SY, Yang AG. Cancer gene therapy targeting cellular apoptosis machinery. Cancer Treat Rev. 2012; 38(7):868–76. [PubMed: 22800735]
43. Zhao H, Spitz MR, Tomlinson GE, Zhang H, Minna JD, Wu X. Gamma-radiation-induced G2 delay, apoptosis, and p53 response as potential susceptibility markers for lung cancer. Cancer Res. 2001; 61(21):7819–24. [PubMed: 11691798]
44. Camplejohn RS, Gilchrist R, Easton D, McKenzie-Edwards E, Barnes DM, Eccles DM, et al. Apoptosis, ageing and cancer susceptibility. Br J Cancer. 2003; 88(4):487–90. [PubMed: 12592359]
45. Imyanitov EN. Gene polymorphisms, apoptotic capacity and cancer risk. Hum Genet. 2009; 125(3):239–46. [PubMed: 19214578]
46. Zheng R, Dahlstrom KR, Wei Q, Sturgis EM. Gamma radiation-induced apoptosis, G2 delay, and the risk of salivary and thyroid carcinomas–a preliminary report. Head Neck. 2004; 26(7):612–8. [PubMed: 15229904]
47. Docherty Z, Georgiou A, Langman C, Kesterton I, Rose S, Camplejohn R, et al. Is chromosome radiosensitivity and apoptotic response to irradiation correlated with cancer susceptibility? Int J Radiat Biol. 2007; 83(1):1–12. [PubMed: 17357435]
48. Klatka J, Rolinski J, Kupisz K, Klonowski S, Skomra D. Expression of bcl-2 protein in lymphocytes of patients with laryngeal carcinoma. Eur Arch Otorhinolaryngol. 1999; 256(6):299–302. [PubMed: 10456279]
49. Zhang L, Zhang W, Wang YF, Liu B, Zhang WF, Zhao YF, et al. Dual induction of apoptotic and autophagic cell death by targeting survivin in head neck squamous cell carcinoma. Cell Death Dis. 2015; 6:e1771. [PubMed: 26018732]
50. Tamm I, Wang Y, Sausville E, Scudiero DA, Vigna N, Oltersdorf T, et al. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res. 1998; 58(23):5315–20. [PubMed: 9850056]
51. Saito T, Kuss I, Dworacki G, Gooding W, Johnson JT, Whiteside TL. Spontaneous ex vivo apoptosis of peripheral blood mononuclear cells in patients with head and neck cancer. Clin Cancer Res. 1999; 5(6):1263–73. [PubMed: 10389908]
52. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tan PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 2010; 363(1): 24–35. [PubMed: 20530316]
53. Munger K, Howley PM. Human papillomavirus immortalization and transformation functions. Virus Res. 2002; 89(2):213–28. [PubMed: 12445661]
54. Chung CH, Gillison ML. Human papillomavirus in head and neck cancer: its role in pathogenesis and clinical implications. Clin Cancer Res. 2009; 15(22):6758–62. [PubMed: 19861444]
55. Mantovani F, Banks L. The human papillomavirus E6 protein and its contribution to malignant progression. Oncogene. 2001; 20(54):7874–87. [PubMed: 11753670]
56. Steller MA, Zou Z, Schiller JT, Baserga R. Transformation by human papillomavirus 16 E6 and E7: role of the insulin-like growth factor 1 receptor. Cancer Res. 1996; 56(21):5087–91. [PubMed: 8895768]
57. Pan H, Griep AE. Temporally distinct patterns of p53-dependent and p53-independent apoptosis during mouse lens development. Genes Dev. 1995; 9(17):2157–69. [PubMed: 7657167]
58. Lacko M, Braakhuis BJ, Sturgis EM, Boedeker CC, Suarez C, Rinaldo A, et al. Genetic susceptibility to head and neck squamous cell carcinoma. Int J Radiat Oncol Biol Phys. 2014; 89(1):38–48. [PubMed: 24725688]

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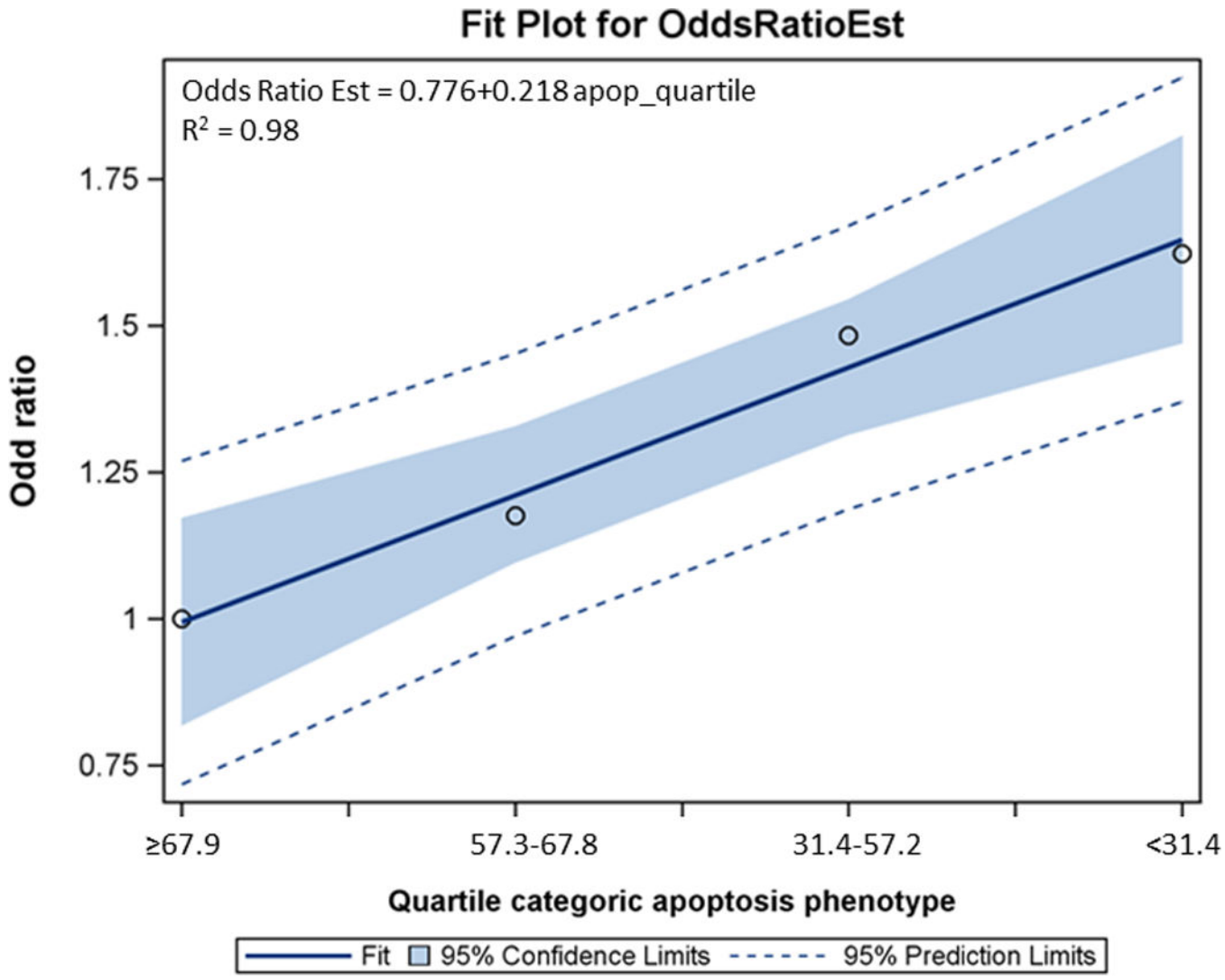

Figure 1.

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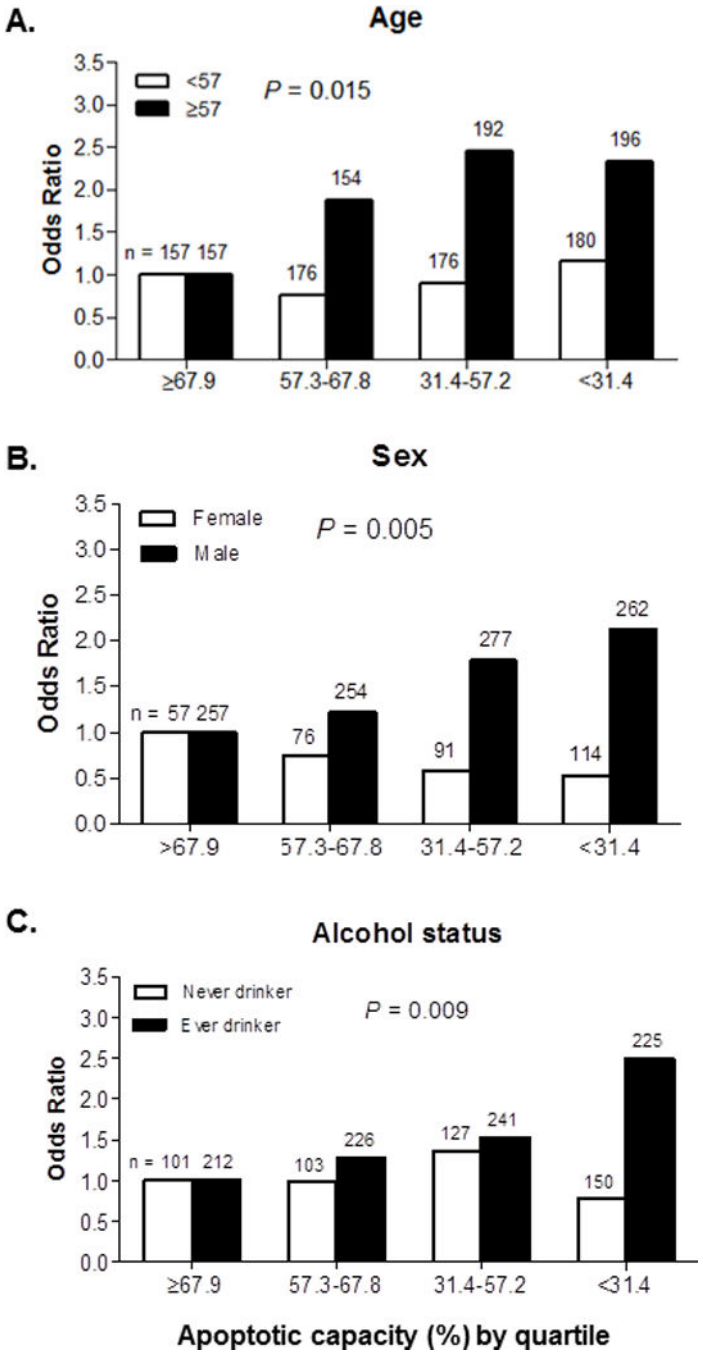

Figure 2.

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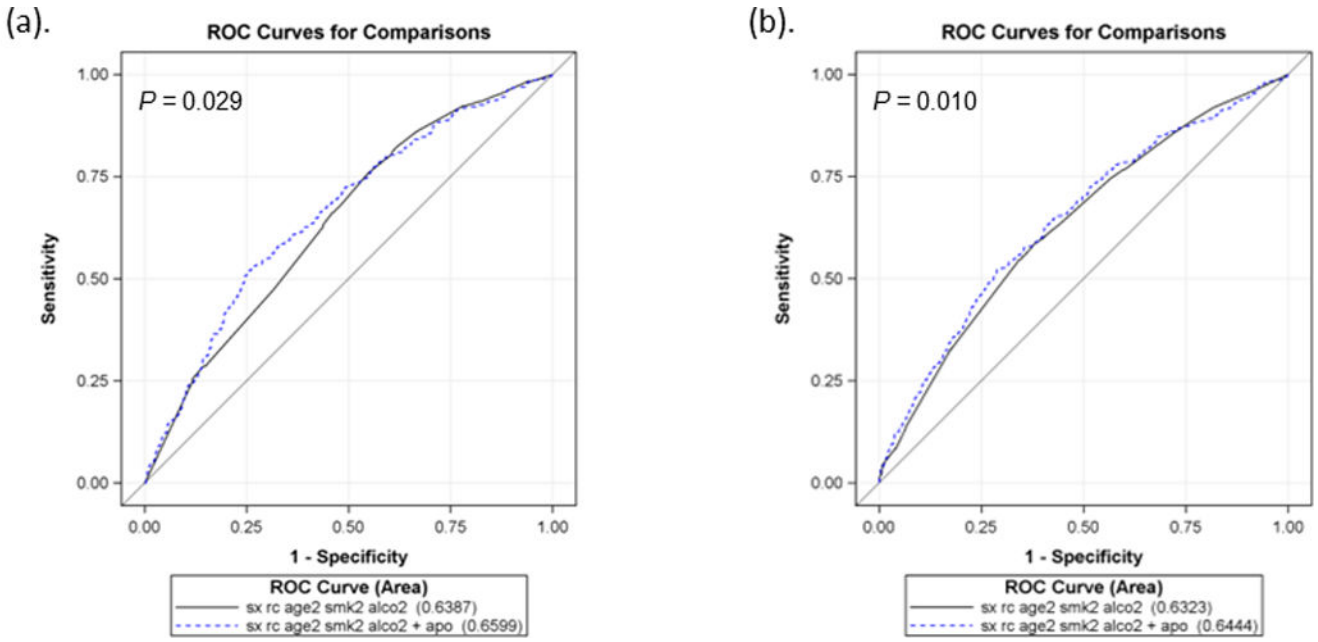

Figure 3.

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Table 1

Characteristics of squamous cell carcinoma of the head and neck (SCCHN) cases and cancer-free controls in a North American population.

Variables	Cases (n = 703)		Controls (n = 685)		*P* [a]
	n	%	*n*	%	
Age (years)					0.745
<57	352	50.1	337	49.2	
≥ 57	351	49.9	348	50.8	
Sex					0.882
Female	170	24.2	168	24.5	
Male	533	75.8	517	75.5	
Race					
Caucasians	647	92.0	647	94.5	0.066
Others	56	8.0	38	5.5	
Asian	5	0.7	1	0.1	
Black	30	4.3	16	2.3	
Hispanic	19	2.7	21	3.1	
American Indian	2	0.3	0	0.0	
Smoking status[b]					< 0.001
Never	250	35.6	363	53.2	
Former	223	31.8	255	37.3	
Current	229	32.6	65	9.5	
Ever	452	64.4	320	46.9	
Alcohol use[b]					<0.0001
Never	187	26.6	294	43.1	
Former	181	25.8	137	20.1	
Current	334	47.6	252	36.9	
Ever	515	73.4	389	57.0	
Tumor site[b]					
Oropharynx	367	52.3			
Non-oropharynx[c]	335	47.7			
T stage[d]					

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Variables	Cases (n = 703)		Controls (n = 685)		*P* [a]
	n	%	*n*	%	
T1	201	28.7			
T2	260	37.0			
T3	123	17.5			
T4	118	16.8			
N stage[d]					
N0	232	33.0			
N1	77	11.0			
N2	381	54.3			
N3	12	1.7			
Overall stage[d]					
I	97	13.8			
II	72	10.3			
III	101	14.4			
IV	432	61.5			

[a] Two-sided χ^2 test.

[b] Missed 1 patients' and 2 controls' information.

[c] Included oral cavity (n=214), hypopharyngeal (n=20) and larynx (n=101) cancer cases.

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Table 2

Apoptotic capacity (AC, %) between squamous cell carcinoma of the head and neck (SCCHN) patients and healthy control subjects in a North American population.

Variable	Cases (AC, %)[a]			Control (AC, %)[a]			P^c
	No.	Mean±SD	P^b	No.	Mean±SD	P^b	
Overall	703	45.9±23.3		685	49.0±23.1		0.002
Age (years)							
<57	352	47.9±23.5		337	48.1±22.4		0.901
≥57	351	43.9±22.9	0.025	348	49.8±23.9	0.326	0.0009[e]
Sex							
Female	170	45.7±23.9		168	41.0±22.8		0.061
Male	533	46.0±23.1	0.916	517	51.6±22.6	<0.0001[e]	<0.0001[e]
Race							
Caucasians	647	46.1±23.4		647	48.9±23.2		0.031
Others	56	43.5±21.2	0.428	38	49.8±22.1	0.814	0.169
Smoking status[d]							
Never	250	44.0±23.7		363	46.5±23.9		0.206
Ever	452	47.0±23.0	0.097	320	51.7±22.0	0.003	0.005
Alcohol status[d]							
Never	187	46.8±23.7		294	44.7±24.2		0.360
Ever	515	45.6±23.1	0.569	389	52.1±21.8	<0.0001[e]	<0.0001[e]
Tumor site[d]							
Oropharynx	367	45.6±23.2					0.024
Non-oropharynx	335	46.4±23.3	0.645				0.096
T stage[d]							
T1	201	43.7±21.7					0.004
T2	260	46.4±23.5					0.122
T3	123	46.2±22.5					0.221
T4	118	48.8±25.7	0.278				0.949
N stage[d]							
N0	232	46.9±23.2		685	49.0±23.1		0.214
N1	77	42.3±23.4					0.016
N2	381	46.3±23.3					0.070
N3	12	44.2±20.5	0.496				0.474
Overall stage[d]							
I	97	42.9±21.7					0.016
II	72	49.9±23.5					0.752
III	101	43.1±22.1					0.016
IV	432	46.7±23.7	0.128				0.110

[a] AC= [100 × (experimental apoptosis − spontaneous apoptosis)/(100 − spontaneous apoptosis)].

[b] *P* values for the differences between subgroups were determined by the Student *t* test.

[c] *P* values for the difference between cases and controls.

[d] Missed 1 patients' and 2 controls' information.

[e] Tests that could pass the Bonferroni threshold (0.05/38 tests =0.0013) for multiple testing correction.

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Table 3

Logistic regression analysis of categorized apoptosis capacity (AC, %) in patients with SCCHN and cancer-free controls in a North American population.

Variable	Cases (n = 703)	Controls (n = 685)	Crude OR (95% CI)	Adjusted OR (95% CI)[a]	P[a]
By median					
≥51.3	301 (42.8)	342 (49.9)	1.00	1.00	0.002
<51.3	402 (57.2)	343 (50.1)	1.33 (1.08–1.65)	1.42 (1.13–1.78)	
By quartile					
≥67.9	142 (20.2)	172 (25.1)	1.00	1.00	0.015
57.3–67.8	159 (22.6)	170 (24.8)	1.13 (0.83–1.55)	1.18 (0.85–1.65)	
31.4–57.2	197 (28.0)	171 (25.0)	1.39 (1.03–1.88)	1.49 (1.08–2.06)	
<31.4	205 (29.2)	172 (25.1)	1.43 (1.06–1.93)	1.61 (1.17–2.22)	
P_{trend}			0.007	0.002	

[a]Obtained from the logistic regression model with adjustment for age, sex, race, smoking and drinking status.

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Table 4

Stratified analysis for association between apoptosis capacity (AC, %) and risk of squamous cell carcinoma of the head and neck (SCCHN) in a North American population

Variable	Cases (n = 703)[a]	Controls (n = 685)[a]	Crude OR (95% CI)	Adjusted OR (95% CI)[b]	P[c]	$P_{interation}$[d]
All subjects:	402/301	343/342	1.33 (1.08–1.65)	1.42 (1.13–1.78)	0.002	
Age						
<57 (years)	186/166	171/166	1.08 (0.80–1.46)	1.17 (0.85–1.61)	0.323	
≥57 (years)	216/135	172/176	1.62 (1.20–2.19)	1.73 (1.25–2.38)	0.0009	0.015
Sex						
Female	95/75	110/58	0.65 (0.42–1.00)	0.66 (0.40–1.06)	0.087	
Male	307/226	233/284	1.65 (1.29–2.11)	1.76 (1.36–2.27)	<0.0001	0.005
Race						
Caucasians	369/278	324/323	1.32 (1.06–1.65)	1.41 (1.12–1.78)	0.004	
Others	33/23	19/19	1.24 (0.53–2.90)	1.98 (0.72–5.47)	0.187	0.697
Smoking status						
Never	153/97	195/168	1.36 (0.98–1.89)	1.36 (0.97–1.90)	0.074	
Ever	248/204	148/172	1.41 (1.06–1.88)	1.48 (1.10–1.99)	0.009	0.566
Alcohol status						
Never	109/78	168/126	1.05 (0.72–1.52)	1.02 (0.70–1.51)	0.905	
Ever	292/223	175/212	1.60 (1.23–2.09)	1.67 (1.27–2.21)	0.0003	0.009
Tumor site						
Oropharynx	215/152	343/342	1.40 (1.09–1.81)	1.59 (1.21–2.07)	0.0008	
Non-oropharynx	186/149	343/342	1.23 (0.95–1.60)	1.26 (0.94–1.68)	0.121	
T stage						
T1	120/81	343/342	1.47 (1.07–2.02)	1.55 (1.12–2.16)	0.009	
T2	148/112	343/342	1.31 (0.98–1.75)	1.46 (1.08–1.97)	0.014	
T3	71/52	343/342	1.33 (0.90–1.97)	1.39 (0.91–2.10)	0.126	
T4	62/56	343/342	1.10 (0.74–1.62)	1.37 (0.89–2.12)	0.158	
T1/T2	268/193	343/342	1.38 (1.09–1.75)	1.47 (1.15–1.89)	0.002	
T3/T4	133/108	343/342	1.21 (0.90–1.63)	1.34 (0.97–1.87)	0.081	
N stage						

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Variable	Cases (n = 703)[a]	Controls (n = 685)[a]	Crude OR (95% CI)	Adjusted OR (95% CI)[b]	P[c]	P interation[d]
N0	130/102	343/342	1.26 (0.94–1.71)	1.32 (0.96–1.82)	0.092	
N1	48/29	343/342	1.61 (0.99–2.61)	1.77 (1.07–2.94)	0.027	
N2	217/164	343/342	1.31 (1.02–1.69)	1.50 (1.15–1.97)	0.003	
N3	6/6	343/342	0.99 (0.32–3.10)	1.19 (0.36–3.91)	0.774	
N0/N1	178/131	343/342	1.34 (1.02–1.76)	1.41 (1.05–1.88)	0.021	
N2/N3	223/170	343/342	1.30 (1.01–1.67)	1.49 (1.14–1.94)	0.003	
Overall stage						
I	60/37	343/342	1.61 (1.04–2.49)	1.60 (1.02–2.50)	0.041	
II	35/37	343/342	0.94 (0.58–1.52)	1.01 (0.61–1.69)	0.956	
III	65/36	343/342	1.76 (1.14–2.72)	1.89 (1.20–2.99)	0.006	
IV	241/191	343/342	1.25 (0.98–1.59)	1.47 (1.13–1.90)	0.004	
I/II	95/74	343/342	1.27 (0.91–1.79)	1.28 (0.90–1.83)	0.167	
III/IV	306/227	343/342	1.33 (1.06–1.67)	1.51 (1.18–1.93)	0.001	

[a] Low AC group (<51.3)/high AC group (≥51.3) using the median apoptotic capacity in controls as the cutoff point value.

[b] Adjusted for age, sex and smoking status in logistic regression models when appropriate.

[c] Obtained from the logistic regression model with adjustment for age, sex, race, smoking and alcohol status.

[d] Interaction *P* values for quartile apoptotic capacity using logistic regression model with adjustment for age, sex, race, smoking and alcohol status.