European Journal of Cancer 72 (2017) 166–176

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Apoptotic capacity and risk of squamous cell carcinoma of the head and neck

Zhensheng Liu [a,b,1], Hongliang Liu [a,b,1], Peng Han [a,b], Fengqin Gao [a,b], Kristina R. Dahlstrom [c], Guojun Li [c,d], Kouros Owzar [a,e], Jose P. Zevallos [f,g], Erich M. Sturgis [c,d], Qingyi Wei [a,b,*]

[a] *Duke Cancer Institute, Duke University Medical Center, Durham, NC 27710, USA*
[b] *Department of Medicine, Duke University School of Medicine, Durham, NC 27710, USA*
[c] *Department of Head and Neck Surgery, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA*
[d] *Department of Epidemiology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA*
[e] *Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC 27710, USA*
[f] *Department of Otolaryngology/Head and Neck Surgery, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA*
[g] *Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA*

Received 6 July 2016; received in revised form 28 September 2016; accepted 20 November 2016
Available online 26 December 2016

KEYWORDS
Biomarker;
Apoptosis;
CPT;
Apoptotic capacity;
Head and neck cancer

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 favour 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 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, subjects with a reduced apoptotic capacity had an increased risk (adjusted odds ratio = 1.42, 95% confidence interval = 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,

* *Corresponding author*: Duke Cancer Institute, Duke University Medical Center, 905 South Lasalle Street, Durham, NC 27710, USA.
E-mail address: qingyi.wei@duke.edu (Q. Wei).
[1] Z. Liu and H. Liu contributed equally to this work.

http://dx.doi.org/10.1016/j.ejca.2016.11.018

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.

1. Introduction

Head and neck cancer is a heterogeneous group of tumours 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 aetiology 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 signalling 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 tumour necrosis factor alpha receptor, 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 signalling leads to the 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]. 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, 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-control study of 703 cases and 685 cancer-free controls of a North American population.