Cancer Epidemiology 35 (2011) e12–e17

Contents lists available at ScienceDirect

Cancer Epidemiology

The International Journal of Cancer Epidemiology, Detection, and Prevention

journal homepage: www.cancerepidemiology.net

Cyclin D1 gene polymorphism, A870G, is associated with an increased risk of salivary gland tumors in the Chinese population

Weijia Liu [a], Enxin Zhu [a], Ru Wang [b], Lihong Wang [c], Lu Gao [a], Xuesong Yang [d], Tingjiao Liu [a,*]

[a] Dalian Medical University, College of Stomatology, Section of Oral Pathology, 116044 Dalian, China
[b] Dalian Medical University, the First Affiliated Hospital, Department of Oral Surgery, 116044 Dalian, China
[c] Graduate School of Northeastern University, Department of Bioengineering, School of Sciences, 110000 Shenyang, China
[d] Dalian Medical University, Department of Biochemistry and Molecular Biology, 116044 Dalian, China

ARTICLE INFO

Article history:
Accepted 16 November 2010
Available online 23 December 2010

Keywords:
Salivary gland tumors
Cyclin D1
Polymorphism

ABSTRACT

Expression of Cyclin D1 is believed to lead to progression through the G1 to S cell cycle checkpoint, and both experimental and pathological evidence suggests that overexpression of this protein may increase the risk of several cancers. The Cyclin D1 A870G polymorphism may modulate expression of the Cyclin D1 protein and is associated with the development of several types of tumor. We investigated the association between the Cyclin D1 A870G polymorphism and susceptibility to salivary gland tumors (SGTs) by PCR-RFLP in 102 Chinese SGT patients and 101 healthy controls. The frequencies of the AG ($p = 0.002$; odds ratio (OR), 3.466) and AA ($p = 0.003$; OR, 3.133) genotypes of Cyclin D1 were significantly higher in patients with SGT than in the healthy controls. The frequencies of these two genotypes were also significantly higher in pleomorphic adenoma (PA) patients ($p = 0.002$; OR, 2.229), compared with the healthy controls. In addition, the expression of Cyclin D1 was found to be significantly higher in PA patients with the AA genotype, compared with PA patients with the GG genotype. Taken together, our results suggested that the Cyclin D1 A870G polymorphism is associated with an increased risk of SGTs in the Chinese population. The Cyclin D1 AA genotype not only increased the risk of PA, but also increased the expression of Cyclin D1 in this type of tumor.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Salivary gland tumors (SGTs) constitute an important area of oral and maxillofacial pathology. SGTs are uncommon, but by no means rare. The annual incidence in the human population worldwide ranges from about 0.4 to 13.5 cases per 100,000. Of these cases, 54–79% represent benign tumors and 21–46% are malignant [1]. Among all patients, the most common tumor type is pleomorphic adenoma (PA), which accounts for about 50% of all tumors. The etiology of SGTs is largely unknown, although several risk factors such as exposure to occupational radiation, family history of cancer and age, are associated with this disease. Elevated levels of cell proliferation have been demonstrated in a high proportion of SGTs [2–5]. Thus, genes that encode regulators of cell proliferation may contribute to the development and progression of SGTs.

Abbreviations: SGT, salivary gland tumor; PA, pleomorphic adenoma; WT, Warthin tumor; MEC, mucoepidermoid carcinoma; ACC, adenoid cystic carcinoma; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

* Corresponding author at: Section of Oral Pathology, College of Stomatology, Dalian Medical University, West Section No. 9, South Road of Lvshun, Dalian 116044, China. Tel.: +86 411 86110395; fax: +86 411 86110398.
E-mail address: tingjiao@dlmedu.edu.cn (T. Liu).

1877-7821/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.canep.2010.11.001

Cyclin D1 protein is expressed in response to mitogenic signals promoting transition through the restriction point in the G1 phase of the cell cycle [6–8]. Increased expression of Cyclin D1 has been associated with increased cell proliferation and poor prognosis in primary laryngeal, esophageal and oral squamous cell carcinomas [9–14]. Similarly, Cyclin D1 overexpression is common in SGTs and has been shown to correlate with reduced five-year, and overall, survival rates in SGT patients [4,5,15,16].

The Cyclin D1 gene is polymorphic with a common A/G substitution at nucleotide 870 in the conserved splice donor region of exon 4 [17]. In heterozygotes, the Cyclin D1a transcript is spliced equally from the Cyclin D1 G870 and A870 alleles, whereas the Cyclin D1b transcript is spliced mainly from the Cyclin D1 A870 allele. The common transcript (Cyclin D1a) interacts with and activates the G1 cyclin-dependent kinases 4 and 6 (CDK4/6). The resulting CDK4/6–Cyclin D1 complex phosphorylates the retinoblastoma (RB) tumor suppressor gene, thereby resulting in cell cycle progression to S-phase. The variant transcript (Cyclin D1b), which results from the polymorphic A allele, encodes a truncated protein isoform with an altered C-terminal domain. Recent studies have shown that this variant transcript demonstrates aberrant nuclear accumulation due to the loss of the phosphorylation site at position 286, required for nuclear export and regulated degradation, suggesting that Cyclin D1b possesses a unique activity not

displayed by Cyclin D1a and is a stronger effector of neoplastic transformation [18,19]. Subsequent studies demonstrated an association between the AA genotype of Cyclin D1 and an increased risk of various human tumors, including colorectal adenomas, esophageal adenocarcinoma, bladder cancer, non-small cell lung cancer and breast cancer [20–32]. In contrast, it was demonstrated that the GG genotype of Cyclin D1 was associated with poorly differentiated squamous cell carcinomas of the head and neck and oral squamous cell carcinoma [33,34]. However, it remains to be determined whether the Cyclin D1 A870G polymorphism effects the development of SGTs.

In this study, we examined the relationship between the Cyclin D1 A870G polymorphism and susceptibility to SGTs. We also studied the influence of Cyclin D1 alleles on protein expression in PA tissue.

2. Materials and methods

2.1. Patients

The study population included a total of 102 individuals (cases) who were diagnosed at The First Affiliated Hospital of Dalian Medical University between October 2008 and February 2010. One hundred and one non-cancer patients from the same hospital and dental clinics were used as the controls. Control subjects were frequency matched to the patients by sex and age. The Human Ethics Committee of the institute has approved the present study.

2.2. Buccal cell collection and genomic DNA extraction

All cases and controls rinsed their mouth thoroughly with distilled water before collection of the buccal cells. A cotton swab was then used to collect the cells from inside the cheeks. A gentle scraping of the inside cheek was done by moving the swab up and down about 10 times on one side of the cheeks. The swab was rinsed in phosphate-buffered saline (PBS) to loosen the cells that had adhered to the cotton. Then this buccal cell suspension was centrifuged and cell pellet were collected. Genomic DNA was isolated from the cell pellet using Oral Mucosa Cell DNA Purification Kit (GENMED SCIENTIFICS INC., U.S.A.).

2.3. Genotyping by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

The genomic fragment containing Cyclin D1 A870G was amplified by a two-step nested PCR with four primers: P1, 5′-ACAGCCTCCTTCCCTCTCTC-3′ (outer forward); P2, 5′-CTGGGACATCACCCTCACTT-3′ (outer reverse); P3, 5′-GTGAAGTTCATTTCCAATCCGC-3′ (inner forward); and P4, 5′-GGGACATCACCCTCACTTAC-3′ (inner reverse). The first- and second-round PCR amplifications were carried out using *Taq* DNA polymerase (Takara, Japan). The first-round PCR was amplified with 0.5 μl of each P1 and P2 and carried out in 50 μl volume containing mixture containing 5 μl genomic DNA, 5 μl PCR buffer, 4 μl dNTPs, and 1.25 μl *Taq* DNA polymerase (Takara, Japan). The first-round PCR product was used as a template DNA for second-round amplification. The second-round PCR was amplified with primers P3 and P4. One DNA fragment of 202 bp was amplified in the first-round PCR; in the second-round PCR, one DNA fragment of 167 bp was amplified.

PCR products were digested with 1.25 U ScrF1 at 37 °C overnight, and visualized by electrophoresis on 3% agarose gels to identify the A870G polymorphic alleles. Samples with the AA genotype produced 1 band (167 bp), samples with the AG genotype produced 3 bands (167 bp, 145 bp, and 22 bp), and samples with the GG genotype produced 2 bands (145 bp and 22 bp).

2.4. Direct DNA sequencing

For confirming Cyclin D1 A870G polymorphism, randomly selected cases comprising of all types of genotypes were sequenced. The PCR products were gel purified and sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Switzerland) and analyzed in the ABI PRISM 3730XL DNA Analyzer.

2.5. Cyclin D1 immunohistochemical staining

Paraffin-embedded tumor material was available in 22 cases from patients with different genotypes. Immunohistochemical staining was performed on 4 μl-thick paraffin sections using the streptavidin–biotin complex technique. The sections were deparaffinized with xylene, rehydrated in graded ethanol, and treated with 0.01 mol/L citrate buffer (pH 6.0) for heat-induced antigen retrieval. The endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide in methanol. After blocking the nonspecific binding sites with 10% normal goat serum, the sections were incubated with optimally diluted (1:50) primary antibody (Clone-SP4, Zeta, CA, USA) overnight at 4 °C in a moist chamber. Immunoreaction was detected using 3,3-diaminobenzidine as the final chromogen. The sections were observed after nuclear staining with hematoxylin. Negative control experiments were carried out by replacing the primary antibodies with PBS. A Cyclin D1-positive oral squamous cell carcinoma was used as an external positive control. Nuclear staining overlapping any cytoplasmic background was considered as an evidence of immunoreactivity. Tumors were scored as follows: score 0, no appreciable staining or staining in $<5\%$ of cancer cells; score 1, tumors with nuclear staining in 5–25% of cancer cells; score 2, tumors with nuclear staining in 25–50% of cancer cells; score 3, strong immunoreactivity of the nucleus in $>50\%$ of cancer cells. Tumors classified as 0 were considered negative, and those scored as 1, 2, and 3 were classified as positive.

2.6. Statistical analysis

Data analysis was performed using software SPSS 13.0 for Windows. Hardy–Weinberg equilibrium analysis was performed to compare observed and expected genotype frequencies using a chi-square test. Cyclin D1 genotypes were categorized into three groups (GG, AG, AA) and as noncarrier (GG) vs. carriers of the A allele (GA, AA). We conducted analysis for all cohorts separately and then combined analysis with adjustment for age and gender. The odds ratio (OR) and 95% confidence intervals (CI) were obtained by Cochran's and Mantel–Haenszel statistics adjusted for age and gender.

3. Results

3.1. Characteristics of the SGT patients

The clinicopathological characteristics of the SGT patients included in this study are summarized in Table 1. They included 45 men and 57 women, with an average age at diagnosis of 53 years. Of the 102 cases studied: 89 cases involved the parotid glands, six cases involved the submandibular glands, one case involved the sublingual gland, and the remaining six cases involved minor salivary glands. The cases comprised of pleomorphic adenomas ($n = 48$ patients), Warthin tumors ($n = 31$ patients), mucoepidermoid carcinomas ($n = 6$ patients), adenoid cystic carcinomas ($n = 4$ patients), and others ($n = 13$ patients). Histopathological diagnoses were made from sections sampled from these tumors, stained with hematoxylin and eosin, by two oral pathologists.

Download English Version:

https://daneshyari.com/en/article/2109150

Download Persian Version:

https://daneshyari.com/article/2109150

Daneshyari.com