CURRENT ONCOLOGY

ORIGINAL ARTICLE

In serum, higher parathyroid hormone but not lower vitamin D is associated with oral squamous cell carcinoma

H. Zhang MD PhD,* H. Lu MD,* C. Shrestha MD,* Y. Feng MD,† Y. Li MD,† J. Peng BS,* Y. Li MD,‡ and Z. Xie MD PhD*

ABSTRACT

Introduction Vitamin D and calcium are known to regulate differentiation and proliferation of keratinocytes; they might potentially have a role in suppressing carcinogenesis in squamous epithelium. Serum parathyroid hormone (PTH) is a sensitive indicator of calcium and vitamin D deficiency, and 25-hydroxyvitamin D [25(OH)D] is an established marker of vitamin D status.

Methods To determine whether levels of 25(OH)D, calcium, or PTH in serum are associated with oral squamous cell carcinoma (OSCC), we examined those parameters in serum collected from 70 patients with OSCC and from an equal number of matched control subjects.

Results The results showed that intact PTH was significantly higher in serum from OSCC patients than in serum from control subjects. However, we observed no significant differences in 25(OH)D or calcium in serum from OSCC patients and from control subjects.

Conclusions We conclude that higher serum PTH, but not lower serum vitamin D or calcium, is associated with OSCC.

Key Words Oral squamous cell carcinoma, parathyroid hormone, 25-hydroxyvitamin D, calcium

Curr Oncol. 2015 Aug;22(4):e259-e263 www.current-oncology.com

INTRODUCTION

Squamous cell carcinoma is a malignant neoplasm arising from transformed keratinocytes. Worldwide, oral squamous cell carcinoma (OSCC), the most common oral cancer, is the 8th most frequent cancer in men and the 14th most frequent in women, accounting for nearly 3% of all cancer cases[1,2]. Morbidity associated with oral cancer has reached up to 40%–50% in India and Pakistan. The 5-year survival rate for oral cancer is less than 50%[2]. Understanding the pathogenesis of this disease will help in the development of new therapeutic strategies.

Vitamin D produced in the skin by exposure to sunlight and obtained from food and supplements has little biologic activity; it requires activation during two hydroxylation steps in liver and kidney. The first step is conversion to 25-hydroxyvitamin D [25(OH)D] by hydroxylation at the 25 position. A subsequent hydroxylation at the 1 position turns the molecule into fully active vitamin D: 1,25-dihydroxyvitamin D [$1,25(OH)_2D$].

The classical role of $1,25(OH)_2D$ is to regulate calcium and bone homeostasis. Together with parathyroid hormone (PTH), $1,25(OH)_2D$ tightly controls calcium concentration in the blood. Conversely, the synthesis and secretion of PTH are inhibited by calcium and by $1,25(OH)_2D$. That negative feedback loop is required to maintain calcium homeostasis in blood.

In addition to regulating calcium homeostasis, $1,25(OH)_2D$ has been demonstrated to regulate cell differentiation and proliferation in a variety of tissues, including squamous epithelium[3]. Keratinocytes (the predominant cell type in the squamous epithelium) synthesize their

Correspondence to: Zhongjian Xie, Department of Endocrinology and Metabolism, The Second Xiangya Hospital, Central South University, 139 Middle Renmin Road, Changsha, Hunan 410011 PRC.
E-mail: Zhongjian.Xie@outlook.com ■ **DOI:** http://dx.doi.org/10.3747/co.22.2259

own $1,25(OH)_2D$ and contain the vitamin D receptor[4]. *In vivo* and *in vitro* studies have indicated that $1,25(OH)_2D$ induces keratinocyte differentiation and inhibits keratinocyte proliferation. In addition to vitamin D, extracellular calcium is a strong inducer of keratinocyte differentiation[5–9]. Vitamin D and calcium might therefore have a role in suppressing carcinogenesis in squamous epithelium.

Although $1,25(OH)_2D$ is the biologically active form of vitamin D, serum 25(OH)D is the measurement of choice to assess vitamin D status. Serum PTH is a sensitive indicator of calcium and vitamin D deficiency. To determine whether serum 25(OH)D, calcium, and PTH are associated with OSCC, we compared levels of calcium, 25(OH)D, and PTH in serum from OSCC patients and from healthy control subjects.

METHODS

Our study recruited 140 subjects (70 with OSCC, 70 healthy control subjects; both groups consisting of 62 men and 8 women) from the Department of Stomatology and the Physical Examination Center of The Second Xiangya Hospital of Central South University in China. The 70 patients with OSCC had been diagnosed pathologically by the Pathology service. Table I presents the demographic and clinical characteristics of the patients and control subjects.

Control subjects were matched to patients on age (±5 years), dates of serum collection (±30 days), number of cigarettes smoked and duration of smoking, amounts and duration of alcohol intake, and duration of betel nut chewing. Body mass of index of the participants was calculated as body weight in kilograms divided by height in metres squared. Figure 1 shows the body mass index results in both groups.

In addition, all participants met these criteria:

- Glomerular filtration rate in the range 100–120 mL/min/1.73 m^2
- No parathyroid disease, rickets, osteomalacia, or sarcoidosis
- Not taking vitamin D, calcium, or any medication affecting calcium or vitamin D metabolism
- Good general condition, normal dietary intake, and no obvious bone metastasis

All participants were volunteers who provided informed consent before joining the study, which was approved by the ethics committee at The Second Xiangya Hospital of Central South University.

Blood samples were taken from the antecubital vein in the morning after an overnight fast. Blood was processed within 1 hour of collection, and serum was isolated and stored at –70°C until analysis. Samples were analyzed in a single batch. Serum 25(OH)D was measured using an enzyme-linked immunosorbent assay (Immunodiagnostic Systems Limited, Boldon, U.K.) performed according to the manufacturer's instructions (detection range: 2.4–144 ng/mL). Plates were analyzed on a microplate absorbance reader (BioTek, Winooski, VT, U.S.A.) at a reading optical density of 450 nm, with a correction wavelength of 620 nm. Serum intact PTH was measured by automated chemiluminescence immunoassay [Siemens

TABLE I Basic characteristics of the study groups

Variable	Patients	Control subjects	*p* Value
Patients (*n*)	70	70	
Mean age (years)	51.5±11.7	53.0±10.7	>0.05
Sex (*n*)			
Men	62	62	
Women	8	8	>0.05
Smoking (*n*)			
Status			
Never	19	19	
Current	51	51	>0.05
Duration			
<20 Years	14	13	
20–29 Years	15	17	
30–39 Years	10	9	
≥40 Years	12	12	>0.05
Frequency			
<9 Times daily	2	4	
10–19 Times daily	4	5	
≥20 Times daily	45	42	>0.05
Alcohol use			
Consumer (*n*)			
Never	45	45	
Current	25	25	>0.05
Duration (*n*)			
<20 Years	5	5	
20–39 Years	15	14	
≥40 Years	5	6	>0.05
Frequency (*n*)			
1–3 Days per week	7	7	
4–7 Days per week	18	18	>0.05
Betel nut use (*n*)			
User			
Never	44	46	
Former	7	6	
Current	19	18	>0.05
Duration			
<20 Years	17	16	
20–39 Years	2	2	
≥40 Years	0	0	>0.05
Frequency			
1–5 Times daily	12	12	
6–10 Times daily	4	4	
>10 Times daily	3	2	>0.05

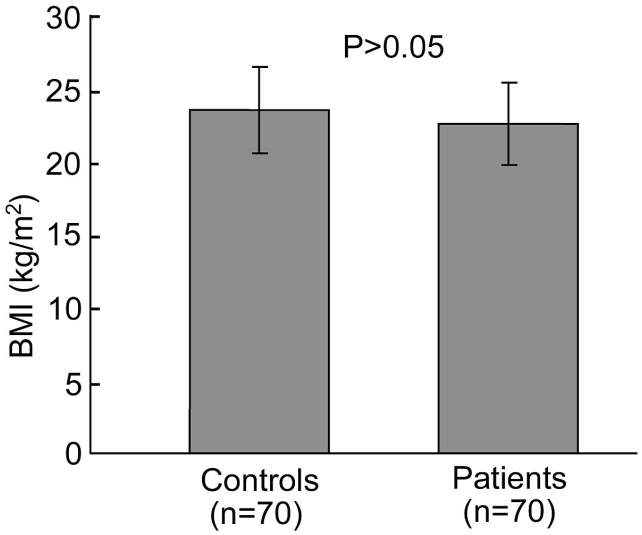


FIGURE 1 Comparison of body mass index (BMI) of patients with oral squamous cell carcinoma and of control subjects. The BMI was calculated by dividing body weight in kilograms by height in metres squared. Data were analyzed using the Student t-test.

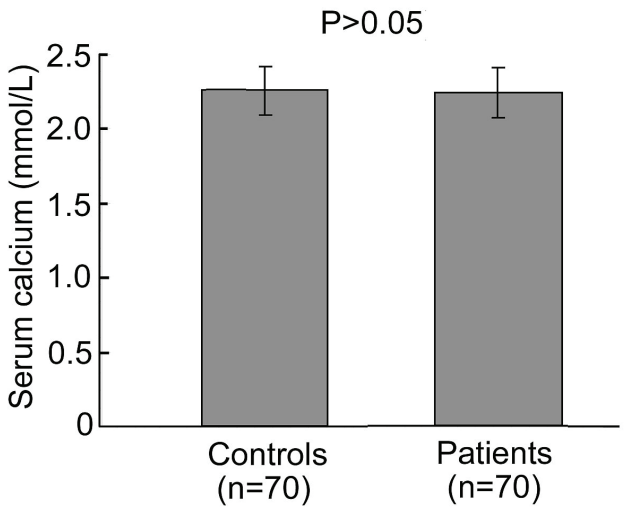


FIGURE 2 Comparison of serum calcium in patients with oral squamous cell carcinoma and in control subjects. Serum calcium was determined using an automated biochemical analyzer. Data were analyzed using the Student t-test.

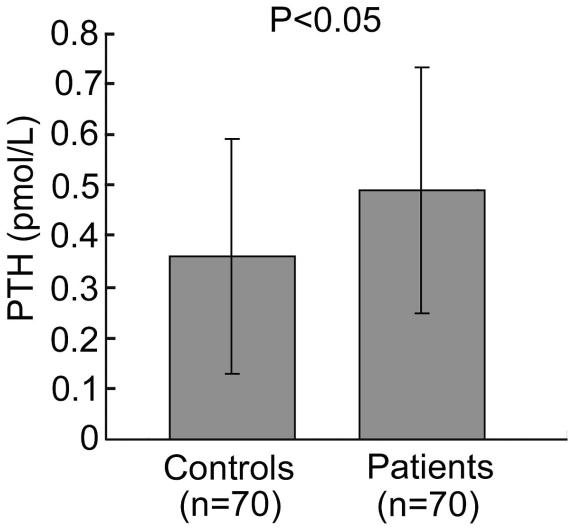


FIGURE 3 Comparison of serum parathyroid hormone (PTH) in patients with oral squamous cell carcinoma and in control subjects. Serum PTH was determined using an automated chemiluminescence immunoassay. Data were log-transformed to achieve normal distribution and analyzed using the Student t-test.

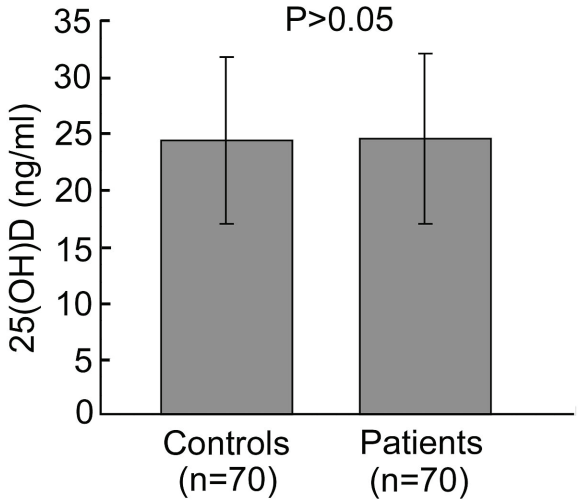


FIGURE 4 Comparison of serum 25-hydroxyvitamin D [25(OH)D] in patients with oral squamous cell carcinoma and in control subjects. Serum 25(OH)D was determined using enzyme-linked immunosorbent assay. Data were analyzed using the Student t-test.

Healthcare Diagnostics, Erlangen, Germany (detection range: 0.265–201 pmol/L)]. All inter- and intra-assay coefficients of variation were less than 10%. Serum calcium, liver function, and renal function were determined using an automatic biochemical analyzer (Abbott Laboratories, North Chicago, IL, U.S.A.).

The Shapiro–Wilk normality test was used to determine the normality of the samples in the patient and control groups. The Student t-test was used to compare continuous variables. The chi-square test was used to compare categorical variables. Results are presented as mean ± standard deviation or median and interquartile range. All p values are two-sided, and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS software application (version 16.0: SPSS, Chicago, IL, U.S.A.).

RESULTS

The oscc patients and control subjects showed no significant differences in smoking, alcohol consumption, and betel nut chewing (Table 1), demonstrating an appropriate

match in the risk factors for OSCC. There was no significant difference in body mass index between the OSCC patients and the control subjects (Figure 1), indicating that the two groups had a fairly similar nutrition status.

As expected, serum calcium was not significantly different in the OSCC patients and in the control subjects (Figure 2), reflecting the tight control of serum calcium by calcium-regulating hormones such as PTH and 1,25(OH)D. With respect to serum PTH, we observed that the concentration of PTH in serum was significantly higher in OSCC patients than in control subjects (Figure 3). To determine whether the higher PTH in OSCC patients was a result of lower vitamin D concentrations, we examined their serum 25(OH)D. The results showed that OSCC patients and control subjects were both vitamin D insufficient. However, serum 25[OH]D was not significantly different between the OSCC patients and the control subjects (Figure 4).

DISCUSSION

Our results show that serum PTH was significantly higher in OSCC patients than in control subjects. Although certain types of cancer—such as lung cancer, hepatocellular carcinoma, and gastric carcinoma—produce PTH[10–16], no available evidence indicates that OSCC produces PTH. The observed increase in PTH in our patients is unlikely to be primary, considering that we observed no increase in serum calcium. Vitamin D status is known to be inversely related to serum PTH. An increase in the secretion of PTH from the parathyroid glands would occur secondary to a decrease in circulating vitamin D. However, the higher level of PTH in OSCC patients in the present study does not appear to be a result of lower circulating vitamin D, because we observed no difference in serum 25(OH)D between OSCC patients and control subjects. Mean serum 25(OH)D in both groups was below 30 ng/mL, which is consistent with previous reports indicating that vitamin D deficiency or insufficiency is prevalent in the Chinese population in almost all age groups and geographic areas[17].

Recent studies have indicated that vitamin D might have anticancer effects. Vitamin D analogs inhibit proliferation of human laryngeal squamous carcinoma cells[18]. Lipworth *et al.*[19] observed a negative correlation between dietary intake of vitamin D and risk of squamous cell carcinoma of the esophagus and oral or pharyngeal cancer. A high prevalence of vitamin D insufficiency has been seen in patients with head-and-neck cancer at diagnosis[20]. However, other studies showed no association between serum 25(OH)D and risk of head-and-neck cancers or upper gastrointestinal cancers[21–23]. Our present data do not support an association between vitamin D status and OSCC.

The parathyroid glands are extremely sensitive to changes in serum calcium and rapidly adjust their PTH secretion. A slight drop in blood calcium (for example, in the case of low calcium intake) is sensed by the parathyroids, resulting in increased secretion of PTH. Elevation in PTH rapidly raises serum calcium to a normal level. It has been shown that the Asian diet is low in calcium[24–26], and calcium supplementation is associated with a reduction in PTH and bone turnover[27]. The higher level of PTH in OSCC patients in the present study might have occurred secondary to a lower calcium intake in a setting in which circulating vitamin D was already low for the individual's requirements.

Recent studies have suggested that PTH stimulates the synthesis of insulin-like growth factor 1 in liver and bone, and acts as an antiapoptotic factor that promotes tumourigenesis[28]. The PTH receptor is expressed in keratinocytes[29,30], and a positive association between the risk of breast cancer and primary hyperparathyroidism has also been reported[31]. However, one study also showed no association between baseline serum PTH and breast cancer risk[32].

We previously demonstrated that high extracellular calcium triggers differentiation of keratinocytes by inducing the formation of the p120–catenin-dependent E-cadherin–beta-catenin complex[7–9]. Lajolo *et al.*[33] showed that mice fed a diet containing 0.12 g calcium glucarate daily had a reduced rate of oral tumours. However, PTH levels were not determined in the mice on the high calcium diet in the study. Those observations suggest that dietary calcium suppresses oral carcinogenesis. However, the underlying mechanism by which dietary calcium suppresses oral carcinogenesis is still unclear. Whether PTH plays a role in promoting carcinogenesis remains a question. More research is needed to address those issues.

CONCLUSIONS

Taken together, our data demonstrate that higher serum PTH, but not lower serum vitamin D or calcium, is associated with OSCC. Intervention studies of strategies to reduce PTH in OSCC patients are required to explore any cause-and-effect relationship.

ACKNOWLEDGMENTS

This work was supported by grants 81072219, 81272973, and 81471055 from the National Natural Science Foundation of China.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare that we have none.

AUTHOR AFFILIATIONS

*Department of Endocrinology and Metabolism, The Second Xiangya Hospital, Central South University, Changsha, PRC; †Department of Stomatology, The Second Xiangya Hospital, Central South University, Changsha, PRC; ‡Stomatological Hospital of Xiangtan, Xiangtan, PRC.

REFERENCES

1. de Camargo Cancela M, Voti L, Guerra-Yi M, *et al.* Oral cavity cancer in developed and in developing countries: population-based incidence. *Head Neck* 2010;32:357–67.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55:74–108.
3. Bikle DD, Oda Y, Xie Z. Calcium and 1,25(OH)2D: interacting drivers of epidermal differentiation. *J Steroid Biochem Mol Biol* 2004;89–90:355–60.
4. Pillai S, Bikle DD, Elias PM. 1,25-Dihydroxyvitamin D production and receptor binding in human keratinocytes varies with differentiation. *J Biol Chem* 1988;263:5390–5.
5. Hennings H, Michael D, Cheng C, Steinert P, Holbrook K, Yuspa SH. Calcium regulation of growth and differentiation of mouse epidermal cells in culture. *Cell* 1980;19:245–54.

6. Pillai S, Bikle DD, Mancianti ML, Cline P, Hincenbergs M. Calcium regulation of growth and differentiation of normal human keratinocytes: modulation of differentiation competence by stages of growth and extracellular calcium. *J Cell Physiol* 1990;143:294–302.
7. Xie Z, Bikle DD. The recruitment of phosphatidylinositol 3–kinase to the E-cadherin–catenin complex at the plasma membrane is required for calcium-induced phospholipase C–gamma1 activation and human keratinocyte differentiation. *J Biol Chem* 2007;282:8695–703.
8. Xie Z, Chang SM, Pennypacker SD, Liao EY, Bikle DD. Phosphatidylinositol-4–phosphate 5–kinase 1alpha mediates extracellular calcium-induced keratinocyte differentiation. *Mol Biol Cell* 2009;20:1695–704.
9. Xie Z, Singleton PA, Bourguignon LY, Bikle DD. Calcium-induced human keratinocyte differentiation requires Src- and Fyn-mediated phosphatidylinositol 3-kinase–dependent activation of phospholipase C-gamma1. *Mol Biol Cell* 2005;16:3236–46.
10. Yoshimoto K, Yamasaki R, Sakai H, *et al.* Ectopic production of parathyroid hormone by small cell lung cancer in a patient with hypercalcemia. *J Clin Endocrinol Metab* 1989;68:976–81.
11. Mahoney EJ, Monchik JM, Donatini G, De Lellis R. Life-threatening hypercalcemia from a hepatocellular carcinoma secreting intact parathyroid hormone: localization by sestamibi single-photon emission computed tomographic imaging. *Endocr Pract* 2006;12:302–6.
12. Abe Y, Makiyama H, Fujita Y, Tachibana Y, Kamada G, Uebayashi M. Severe hypercalcemia associated with hepatocellular carcinoma secreting intact parathyroid hormone: a case report. *Intern Med* 2011;50:329–33.
13. Nakajima K, Tamai M, Okaniwa S, *et al.* Humoral hypercalcemia associated with gastric carcinoma secreting parathyroid hormone: a case report and review of the literature. *Endocr J* 2013;60:557–62.
14. Nielsen PK, Rasmussen AK, Feldt-Rasmussen U, Brandt M, Christensen L, Olgaard K. Ectopic production of intact parathyroid hormone by a squamous cell lung carcinoma *in vivo* and *in vitro*. *J Clin Endocrinol Metab* 1996;81:3793–6.
15. Uchimura K, Mokuno T, Nagasaka A, *et al.* Lung cancer associated with hypercalcemia induced by concurrently elevated parathyroid hormone and parathyroid hormone-related protein levels. *Metabolism* 2002;51:871–5.
16. Eid W, Wheeler TM, Sharma MD. Recurrent hypercalcemia due to ectopic production of parathyroid hormone-related protein and intact parathyroid hormone in a single patient with multiple malignancies. *Endocr Pract* 2004;10:125–8.
17. Zhang W, Stoecklin E, Eggersdorfer M. A glimpse of vitamin D status in mainland China. *Nutrition* 2013;29:953–7.
18. Lu L, Qiu J, Liu S, Luo W. Vitamin D3 analogue EB1089 inhibits the proliferation of human laryngeal squamous carcinoma cells via p57. *Mol Cancer Ther* 2008;7:1268–74.
19. Lipworth L, Rossi M, McLaughlin JK, *et al.* Dietary vitamin D and cancers of the oral cavity and esophagus. *Ann Oncol* 2009;20:1576–81.
20. Orell-Kotikangas H, Schwab U, Osterlund P, Saarilahti K, Makitie O, Makitie AA. High prevalence of vitamin D insufficiency in patients with head and neck cancer at diagnosis. *Head Neck* 2012;34:1450–5.
21. Meyer F, Liu G, Douville P, *et al.* Dietary vitamin D intake and serum 25-hydroxyvitamin D level in relation to disease outcomes in head and neck cancer patients. *Int J Cancer* 2011;128:1741–6.
22. Arem H, Weinstein SJ, Horst RL, *et al.* Serum 25-hydroxyvitamin D and risk of oropharynx and larynx cancers in Finnish men. *Cancer Epidemiol Biomarkers Prev* 2011;20:1178–84.
23. Abnet CC, Chen Y, Chow WH, *et al.* Circulating 25-hydroxyvitamin D and risk of esophageal and gastric cancer: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. *Am J Epidemiol* 2010;172:94–106.
24. Ge KY, Chang SY. Dietary intake of some essential micronutrients in China. *Biomed Environ Sci* 2001;14:318–24.
25. Qu N, Gao J, Li W, Tian Y. The Chinese total diet study in 1992—macro elements and trace elements [Chinese]. *Wei Sheng Yan Jiu* 1997;26:117–21,125.
26. Tian HG, Nan Y, Hu G, *et al.* Dietary survey in a Chinese population. *Eur J Clin Nutr* 1995;49:26–32.
27. Lau EM, Woo J, Lam V, Hong A. Milk supplementation of the diet of postmenopausal Chinese women on a low calcium intake retards bone loss. *J Bone Miner Res* 2001;16:1704–9.
28. McCarty MF. Parathyroid hormone may be a cancer promoter—an explanation for the decrease in cancer risk associated with ultraviolet light, calcium, and vitamin D. *Med Hypotheses* 2000;54:475–82.
29. Errazahi A, Bouizar Z, Lieberherr M, Souil E, Rizk-Rabin M. Functional type I PTH/PTHrP receptor in freshly isolated newborn rat keratinocytes: identification by RT-PCR and immunohistochemistry. *J Bone Miner Res* 2003;18:737–50.
30. Errazahi A, Lieberherr M, Bouizar Z, Rizk-Rabin M. PTH-1R responses to PTHrP and regulation by vitamin D in keratinocytes and adjacent fibroblasts. *J Steroid Biochem Mol Biol* 2004;89–90:381–5.
31. Michels KB, Xue F, Brandt L, Ekbom A. Hyperparathyroidism and subsequent incidence of breast cancer. *Int J Cancer* 2004;110:449–51.
32. Almquist M, Bondeson AG, Bondeson L, Malm J, Manjer J. Serum levels of vitamin D, PTH and calcium and breast cancer risk—a prospective nested case–control study. *Int J Cancer* 2010;127:2159–68.
33. Lajolo C, Sgambato A, Maiorano E, *et al.* Calcium glucarate inhibits DMBA-induced oral carcinogenesis in the hamster: histomorphometric evaluation. *Anticancer Res* 2010;30:843–9.