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ORIGINAL ARTICLE

Synergic effect of curcumin or lycopene with irradiation upon oral squamous cell carcinoma cells

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OBJECTIVE: An *in vitro* study was carried out to evaluate the effect of curcumin, lycopene, and irradiation upon oral squamous cell carcinoma (OSCC).
MATERIALS AND METHODS: Curcumin and lycopene were administrated at doses of 3, 4.25, 5.50, and 6.75 μM in PE/CA-PJ15 OSCC cultures irradiated with different doses (1, 2.5, and 5 Gy), followed by evaluation of the effects upon cell viability, apoptosis, and migration after 24, 48, and 72 h of incubation.
RESULTS: The application of curcumin or lycopene to the tumor cells during 24, 48, and 72 h without irradiation exerted an inhibitor effect upon cell viability and increased cell apoptosis. The maximum reduction in cell viability and the peak apoptotic effect was recorded with the 5.50 and 6.75 μM doses, for both curcumin and lycopene. Likewise, curcumin and lycopene exerted a synergic effect upon both variables on applying irradiation. Lastly, the 5.50 and 6.75 μM drug doses, together with 5 Gy of irradiation, yielded the greatest decrease in cell migration capacity with both curcumin and lycopene.
CONCLUSIONS: Curcumin and lycopene increase cytotoxic activity in the PE/CA-PJ15 cell line and reduce cell migration capacity, while the combination of curcumin or lycopene with irradiation exerts a synergic effect.
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Keywords: curcumin; lycopene; squamous cell carcinoma; irradiation; *in vitro* cell line

Introduction

Head and neck cancer is the fifth leading cause of cancer and the sixth main cause of cancer mortality in the world. More than 500 000 new cases are diagnosed worldwide each year, including 100 000 cases in Europe alone. Oral squamous cell carcinoma (OSCC) is the most common head and neck cancer and accounts for approximately 3% of all newly diagnosed cancer cases worldwide (Reid *et al*, 2000; Parkin *et al*, 2005). Despite recent advances in treatment, including new surgical techniques and radiotherapy and chemotherapy protocols, the long-term survival of patients with OSCC has remained unsatisfactory for the past three decades; in effect, the 5-year survival rate remains low at approximately 50% (Petersen, 2009).

In this situation, the development of an oral cancer-specific, anticancer drug is needed. Traditional natural products have recently been extensively examined because they show few side effects and have powerful antioxidative, antiinflammatory, and cancer-preventing properties (Aggarwal and Shishodia, 2006). For example, half a century ago, curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione] (a phenolic compound isolated from the dietary plant *Curcumina longa*) and its family of related compounds were reported to have antibacterial effects, and many researchers have subsequently studied their antioxidant and anticancer properties. Curcumin is one of the main components of the Indian curry spice turmeric, and traditional Indian medicine claims the use of its powder against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis (Ammon *et al*, 1992). Recently, curcumin has also been shown to possess potent antineoplastic activity against a number of tumors including prostate, breast, colon, and oral cancer (Baselga and Averbuch, 2000; Newman *et al*, 2003; Aggarwal *et al*, 2004a,b; Rogers *et al*, 2005; Srinivasan *et al*, 2006; Jagetia, 2007; Strimpakos and Sharma, 2008; Khafif *et al*, 2009). Although the precise mechanisms of action of curcumin remain unclear, the existing evidence suggests that it suppresses cancer cell growth largely by cell proliferation and survival downregulating pathways. In this context, curcumin inhibition of the NF-*k*B as well as the PI3K-Akt pathways of cell survival has been well documented. As the inhibition of NF-*k*B and PI3K-Akt signaling is often associated with an increased apoptotic index in many cell types, the anticancer effects of curcumin would also involve the activation of the extrinsic and/or intrinsic

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pathways of apoptosis (Ramachandran *et al*, 2005; Reuter *et al*, 2008). Depending on the cell type, and besides the inhibition of NF-*k*B, curcumin may use other molecular mechanisms such as the downregulation of c-*myc*, cyclin D1, and protein tyrosine kinases (Aggarwal *et al*, 2003). Interestingly, the expression of cyclin D1 is also downregulated by the inhibition of eIF4E in head and neck cancer cells (Oridate *et al*, 2005). Earlier studies have shown curcumin treatment to result in the suppression of head and neck squamous cell carcinoma (HNSCC) growth both *in vitro* (Chakravarti *et al*, 2006) and *in vivo* (LoTempio *et al*, 2005).

In this context, lycopene (ψ, ψ-carotene), one of more than 600 carotenoids synthesized by plants and photosynthetic microorganisms, is a tetraterpene hydrocarbon containing 40 carbon atoms and 56 hydrogen atoms, with a molecular weight of 536. Lycopene is the most abundant carotenoid in tomatoes (*Lycopersicon esculentum* L.), with concentrations ranging from 0.9 to 4.2 mg/100 g depending upon the variety [21]. Although long used as a food colorant, lycopene has recently been the subject of intense research with respect to its antioxidant activity and potential role in the prevention and treatment for chronic diseases including cancers, cardiovascular disorders, neurodegenerative diseases, and bone disorders (Clinton, 1998; Story *et al*, 2010). More recently, epidemiological studies, as well as *in vitro* studies, animal studies, and clinical trials have suggested that lycopene has some beneficial effects in the treatment for certain diseases of the oral cavity – including oral cancer and precancerous lesions (Gupta *et al*, 1998; Livny *et al*, 2002; Mayne *et al*, 2004; Singh *et al*, 2004; Kumar *et al*, 2007). Although the epidemiological evidence of the role of lycopene in cancer prevention and treatment is persuasive, this mechanism of bioactivity remains to be proven. Unlike some other carotenoids, lycopene lacks the β-ionone ring structure and provitamin A activity (Stahl and Sies, 1996). Therefore, its biological effects have been attributed to mechanisms other than those of vitamin A. Two major hypotheses have been proposed to explain the bioactivities of lycopene: antioxidative and non-oxidative effects. Oxidative stress caused by reactive oxygen species (ROS) can result in damage to macromolecules such as proteins, carbohydrates, and DNA and may be involved in carcinogenesis. As a quencher of single oxygen and a scavenger of free radicals, lycopene is able to protect against oxidative stress (Van Breemen and Pajkovic, 2008). The quenching capacity of a carotenoid depends primarily on the number of conjugated double bonds it contains, which makes lycopene one of the most efficient antioxidants, with a singlet-oxygen-quenching ability twice as high as that of β-carotene and 10 times higher than that of vitamin E (Di Mascio *et al*, 1989; Ukai *et al*, 1994). The postulated non-oxidative anticarcinogenic mechanisms of action of lycopene also include an increase in gap-junction communication, gene function regulation, antiproliferation and pro-differentiation activities, the induction of apoptosis, the modulation of carcinogen metabolizing enzymes, and the modulation of immune function (Agarwal and Rao, 2000; Heber and Lu, 2002; Van Breemen and Pajkovic, 2008).

Lastly, studies of plant extracts and phytochemicals as modifiers of irradiation effects represent a new field of research. In effect, humans consume a variety of phytochemicals that afford protection from irradiation exposure. In this context, it is necessary to assess the protective action of such commonly used phytochemicals and exploit their possible application in cancer irradiation therapy as an alternative source of non-toxic radioprotectors. Radiosensitization has been extensively studied with different chemotherapy drugs (cisplatin, 5-fluorouracil, taxol) either prior to irradiation or as concomitant treatment for patients with head and neck malignances (Pignon *et al*, 2000; Moreno-Jiménez *et al*, 2010).

The aim of the present study was to evaluate the effect of curcumin, lycopene, and irradiation upon oral OSCC.

Material and methods

Cell line

We used the PE/CA-PJ15 human oral squamous carcinoma cell line (European Collection of Cell Cultures) cultured in Iscove's modified Dulbecco's modified Eagle medium (DMEM) (IMDM) supplemented with 10% fetal calf serum (FCS), 1% penicillin, and 1% streptomycin (full medium) at 37°C, in an atmosphere of 95% oxygen and 5% CO_2. The medium (IMDM), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), dimethylsulfoxide (DMSO), curcumin, and lycopene were obtained from Sigma-Aldrich® (Sigma-Aldrich Chemistry, S.A., Madrid, Spain).

Drug preparation

Curcumin and lycopene (Figure 1) were dissolved in 0.5% DMSO, with 1 mg ml^{-1} of curcumin or lycopene being used a stock solution. The working solutions were diluted with sterile distilled water. All manipulations with curcumin and lycopene were performed under subdued light, the dose range being 3, 4.25, 5.50, and 6.75 μM of curcumin or lycopene.

Irradiation

Irradiation of the cells was performed using a linear accelerator (Yxlon Smart; Krautkrämer-Forster Spanish S.A,

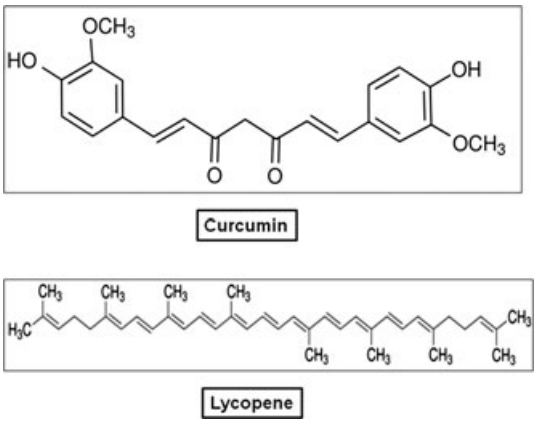

Figure 1 Chemical composition of curcumin and lycopene

Madrid, Spain). The machine was calibrated for the field size of interest using both special small ionization chambers and thermo-luminescence dosimetry. Single irradiation doses of 1, 2.5, and 5 Gy were administrated. The cells were irradiated in 96-microwell plates.

Cell viability test (MTT)
We used the technique described by Carmichael *et al* (1987a,b), adapted to our culture conditions for the quantification of cell viability. The cells were cultured at a density of 5×10^3 cells per well in 96-microwell plates, after which curcumin or lycopene was added at different concentrations (3, 4.25, 5.50, and 6.75 μM), and 15 min after plate irradiation (1, 2.5, and 5 Gy).

At different time points after the start of treatment (24, 48, and 72 h), the medium was eliminated and the cells were incubated with MTT (Sigma-Aldrich Chemistry, S.A.) (1 mg ml^{-1}) during 4 h, after which the non-metabolized MTT was discarded and 100 μl of DMSO was added to each well. We measured the absorbance in each well with an enzyme-linked immunosorbent assay (ELISA), using a Multiskan MCC/340P plate spectrophotometer at a reading wavelength of 570 nm and a reference wavelength of 690 nm. This experiment was performed in triplicate.

Apoptosis (Histone/DNA fragment ELISA)
The cell apoptosis ELISA detection kit was used to detect apoptosis in cells treated with curcumin or lycopene and irradiation according to the protocol of the manufacturer. Briefly, cells were seeded in 96-well plates at a density of 1×10^4 cells per well for 24 h, added with the medium containing different concentrations (3, 4.25, 5.50, and 6.75 μM) of curcumin or lycopene, and 15 min after plate irradiation (1, 2.5, and 5 Gy). After 24, 48, or 72 h, we transferred the cytoplasm of the control and treatment groups to the 96-well plate peridiumed with streptavidin and incubated with the biotinylated histone antibody and peroxidase-tagged mouse anti-human DNA for two hours at room temperature. The absorbance at 405 nm was measured with an EXL-800 type Enzyme-Linked Immunosorbent apparatus. This experiment was performed in triplicate.

Migration (scratch wound healing)
Scratch wounds were generated in confluent monolayers of cells using a sterile 200-μl pipette tip (Gerhartz *et al*, 2007). After washing away suspended cells with PBS, the culture medium was changed and added at different concentrations (3, 4.25, 5.50, and 6.75 μM), 15 min after plate irradiation (1, 2.5, and 5 Gy).

Migration into the wound space was photographed using an inverted microscope equipped with a digital camera at the time of the initial wound and at time intervals up to 4 and 8 h postwounding. The relative distances between edges of the injured monolayer were obtained via pixel counts at a minimum of 10 sites of the wound, using MIP-4® image software (CID, Barcelona, Spain) and applying the formula: migration distance = initial distance of free-of-cells space – distance at 4 or 8 h of free-of-cells space (Valster *et al*, 2005). This experiment was performed in triplicate.

Statistical analysis
Data were analyzed using the SPSS version 12.0 statistical package (SPSS® Inc., Chicago, IL, USA). A descriptive study was made of each variable. The associations between different quantitative variables were studied using the Student's *t*-test for two independent samples and one-way analysis of variance (ANOVA) for more than two samples, verifying in each case whether the variances were homogeneous. Statistical significance was accepted for $P \leq 0.05$.

Results

Effects of curcumin, lycopene, and irradiation upon PE/CA-PJ15 cell viability
24 h of incubation. At a concentration of 5.50 μM, curcumin induced a greater decrease in cell viability than lycopene, at all the irradiation doses used, with statistically significant differences at 0 and 2.5 Gy ($P = 0.032$ and 0.007, respectively) (Figure 2a). However, at a concentration of 6.75 μM, no statistically significant differences were observed between curcumin and lycopene (Figure 2b).

48 h of incubation. At a concentration of 5.50 μM, curcumin induced a greater decrease in cell viability than lycopene at the irradiation doses of 0, 2.5, and 5 Gy – the differences being statistically significant ($P = 0.017$, $P < 0.001$, and $P < 0.001$, respectively) (Figure 2c). In contrast, at a concentration of 6.75 μM, significant differences between curcumin and lycopene were only observed at an irradiation dose of 2.5 Gy ($P < 0.001$) (Figure 2d).

72 h of incubation. At a concentration of 5.50 μM, significant differences were observed at irradiation doses of 1 and 2.5 Gy ($P = 0.002$ and $P = 0.048$, respectively), with lesser cell viability on applying lycopene (Figure 2e). Lastly, at a concentration of 6.75 μM, statistically significant differences were observed at irradiation doses of 0, 1, and 2.5 Gy ($P = 0.001$, $P < 0.001$ and $P = 0.001$, respectively) (Figure 2f).

Effects of curcumin, lycopene and irradiation upon PE/CA-PJ15 cell apoptosis
24 h of incubation. At concentrations of both 5.50 and 6.75 μM, lycopene induced significantly greater cell apoptosis than curcumin. The only exception corresponded to a concentration of 5.50 μM and an irradiation dose of 5 Gy, where significantly greater apoptosis was observed on applying curcumin ($P = 0.007$) (Figure 3a,b).

48 h of incubation. At a concentration of 5.50 μM, increased cell apoptosis was observed with curcumin, at all the irradiation doses used, although statistical significance was only reached with the 5 Gy dose ($P = 0.004$) (Figure 3c). In contrast, at a concentration of 6.75 μM, significant differences were recorded at all irradiation doses except the 1 Gy dose, where lycopene induced greater apoptosis ($P = 0.092$) (Figure 3d).

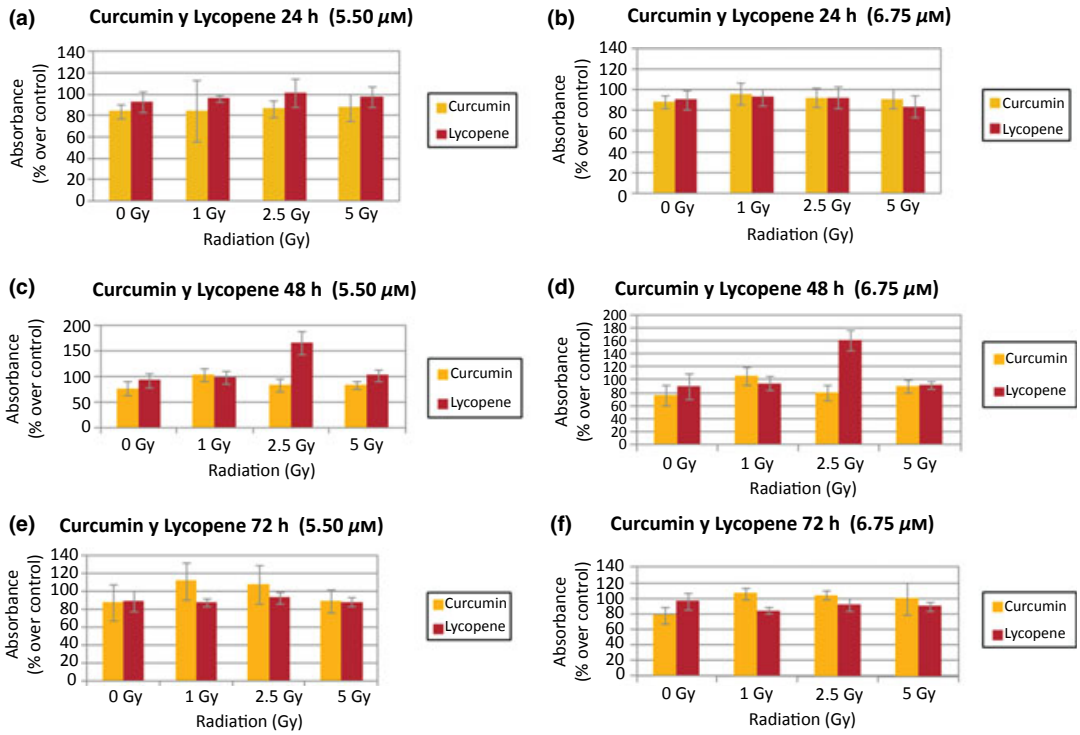

Figure 2 Effects of curcumin, lycopene, and irradiation upon PE/CA-PJ15 cell viability. (**a**) and (**b**) at 24 h of incubation, (**c**) and (**d**) at 48 h of incubation, and (**e**) and (**f**) at 72 h of incubation (**a**: 0 Gy, $P = 0.032$; 1 Gy, $P = 0.236$; 2.5 Gy, $P = 0.007$; 5 Gy, $P = 0.067$. **b**: 0 Gy, $P = 0.520$; 1 Gy, $P = 0.435$; 2.5 Gy, $P = 0.963$; 5 Gy, $P = 0.123$. **c**: 0 Gy, $P = 0.017$; 1 Gy, $P = 0.297$; 2.5 Gy, $P < 0.001$; 5 Gy, $P < 0.001$. **d**: 0 Gy, $P = 0.084$; 1 Gy, $P = 0.073$; 2.5 Gy, $P < 0.001$; 5 Gy, $P = 0.528$. **e**: 0 Gy, $P = 0.851$; 1 Gy, $P = 0.002$; 2.5 Gy, $P = 0.048$; 5 Gy, $P = 0.804$. **f**: 0 Gy, $P = 0.001$; 1 Gy, $P < 0.001$; 2.5 Gy, $P = 0.001$; 5 Gy, $P = 0.181$)

72 hours of incubation. Maximum apoptosis was recorded at a concentration of 6.75 μM and an irradiation dose of 5 Gy, with statistically significant differences in favor of lycopene (Figure 3e,f).

Effects of curcumin, lycopene, and irradiation upon PE/CA-PJ15 cell migration

Both curcumin and lycopene reduced cell migration capacity–this effect increasing in magnitude with the concentration used, both 4 and 8 h after scratch induction. Maximum reduction in cell migration capacity was observed at concentrations of 5.50 and 6.75 μM, and with an irradiation dose of 5 Gy (Figures 4 and 5).

Discussion

The agents most commonly used in chemotherapy regimens for head and neck cancer are cisplatin or carboplatin, often administered in combination with taxanes and/or 5-fluorouracil – the duration of therapy being limited due to toxic effects (Moreno-Jiménez *et al*, 2010). Consequently, new therapeutic strategies need to be identified and evaluated in preclinical models before entering clinical trials. In this situation, development of an oral cancer-specific, anticancer drug is needed.

Recently, *in vitro* and *in vivo* studies (in experimental animal models) have shown curcumin to have important chemopreventive and chemotherapeutic effects (Aggarwal *et al*, 2003) against a number of tumors. In addition to its antioxidant activity, curcumin has many pharmacological targets in different cancers. This explains the extensive interest in the clinical development of this compound as a cancer chemopreventive and/or chemotherapeutic agent, as evidenced by the conduction of phase I clinical trials and current enrollment in phase II clinical trials (Johnson and Mukhtar, 2007). The mechanisms whereby curcumin exerts its antioxidative effects are well known and are characterized by its influence upon lipid peroxidation, which moreover reduces inflammation and would explain its important preventive and curative effects upon cardiocirculatory diseases (Reddy and Lokesh, 1992). In contrast, the precise mechanisms of action of curcumin in relation to cancer remain to be established. The existing evidence suggests that it suppresses cancer cell growth largely by cell proliferation and survival downregulating pathways.

In our study, curcumin exerted a cytotoxic effect upon the PE/CA-PJ15 tumor cells. The magnitude of this effect was directly proportional to the curcumin concentration used – the greatest reduction in cell viability and increase in apoptosis being achieved with doses of 5.50 and 6.75 μM. In this context, López-Jornet *et al* (López-Jornet *et al*, 2011) examined the effect of curcumin with and without irradiation upon cell viability in this same OSCC cell line, using curcumin doses of 3, 3.75, 4.50, and 5.25 μM. Their results reflected a greater reduction in cell viability (with and without irradiation) at a concentration of 5.25 μM. Similar results have been published by other

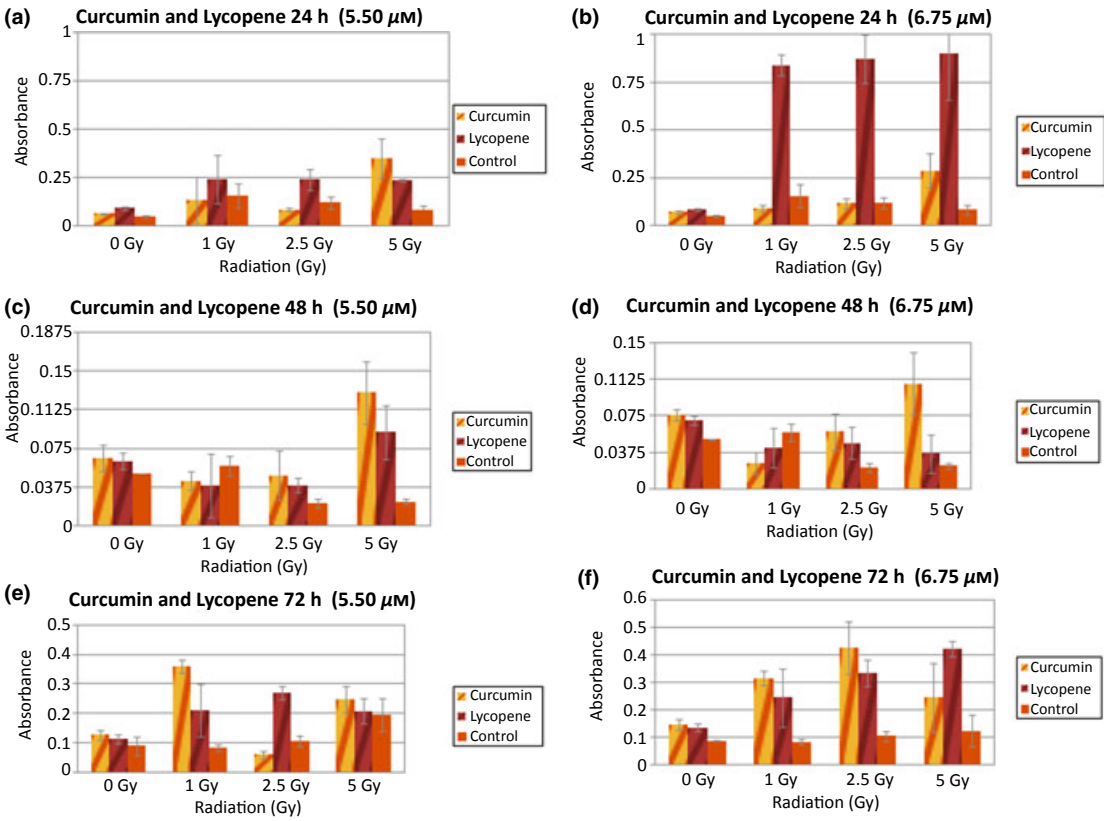

Figure 3 Effects of curcumin, lycopene, and irradiation upon PE/CA-PJ15 cell apoptosis. (**a**) and (**b**) at 24 h of incubation, (**c**) and (**d**) at 48 h of incubation, and (**e**) and (**f**) at 72 h of incubation (**a**: 0 Gy, $P = 0.001$; 1 Gy, $P = 0.468$; 2.5 Gy, $P = 0.004$; 5 Gy, $P = 0.007$. **b**: 0 Gy, $P = 0.006$; 1 Gy, $P < 0.001$; 2.5 Gy, $P \leq 0.001$; 5 Gy, $P = 0.001$. **c**: 0 Gy, $P = 0.163$; 1 Gy, $P = 0{,}500$; 2.5 Gy, $P = 0.153$; 5 Gy, $P = 0.004$. **d**: 0 Gy, $P = 0.001$; 1 Gy, $P = 0.092$; 2.5 Gy, $P = 0.054$; 5 Gy, $P = 0.007$. **e**: 0 Gy, $P = 0.032$; 1 Gy, $P = 0.002$; 2.5 Gy, $P < 0.001$; 5 Gy, $P = 0.434$. **f**: 0 Gy, $P = 0.006$; 1 Gy, $P = 0.011$; 2.5 Gy, $P = 0.002$; 5 Gy, $P = 0.033$)

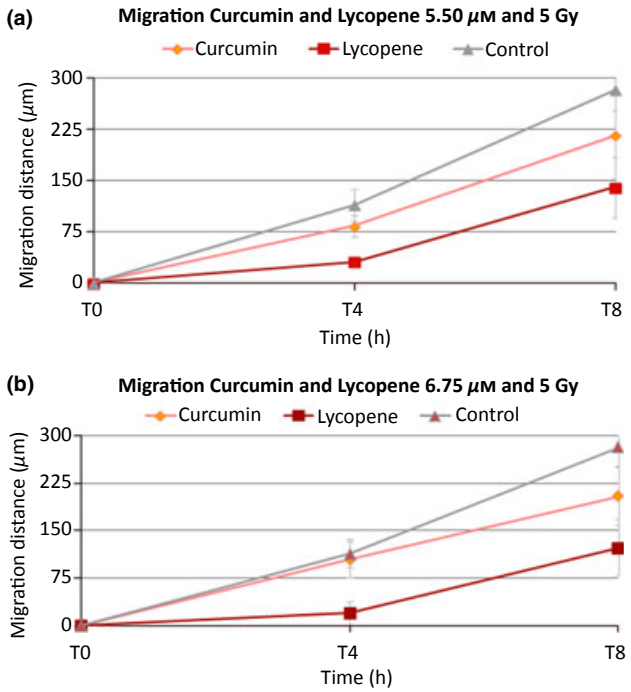

Figure 4 Effects of curcumin and irradiation upon PE/CA-PJ15 cell migration (**a**: T4, $P < 0.001$; T8, $P = 0.008$. **b**: T4, $P = 0.001$; T8, $P = 0.001$)

authors (Clark *et al*, 2010) with different squamous cell carcinoma cell lines, in which a curcumin dose of 10 μM induced significant inhibition of cell proliferation and an increase in apoptosis (Li *et al*, 2005). Lastly, our results following curcumin application revealed a potent inhibitory effect upon OSCC cell migration capacity. It is now known that cancer cell migration is a highly regulated process that permits dissemination from the primary tumor. The invasive phenotype in malignant cells can be inhibited by decreasing matrix metalloproteinases (MMPs) (Deryugina and Quigley, 2006). MMP-9 aberrant expression in HNSCC has been linked to enhanced tumor invasion or metastasis (O-charoenrat *et al*, 2001; Nathan *et al*, 2002). Curcumin has been found to reduce migration and invasion of endothelial cells, non-small cell lung cancer, colorectal cancer, and hepatocellular carcinoma cells and inhibits the migration of vascular smooth muscle cells when exposed to the same concentrations of curcumin, by blocking MMP-9 (Kunjnumakkara *et al*, 2008; Clark *et al*, 2010).

Regarding lycopene (the most abundant carotenoid in tomatoes), there are epidemiological data to support the hypothesis that it exerts an independent protective effect against the development of head and neck cancer (Mackerras *et al*, 1988; Tavani *et al*, 1994). Free radicals such as ROS and reactive nitrogen species (RNS) have been implicated in the development of oral cancer in many

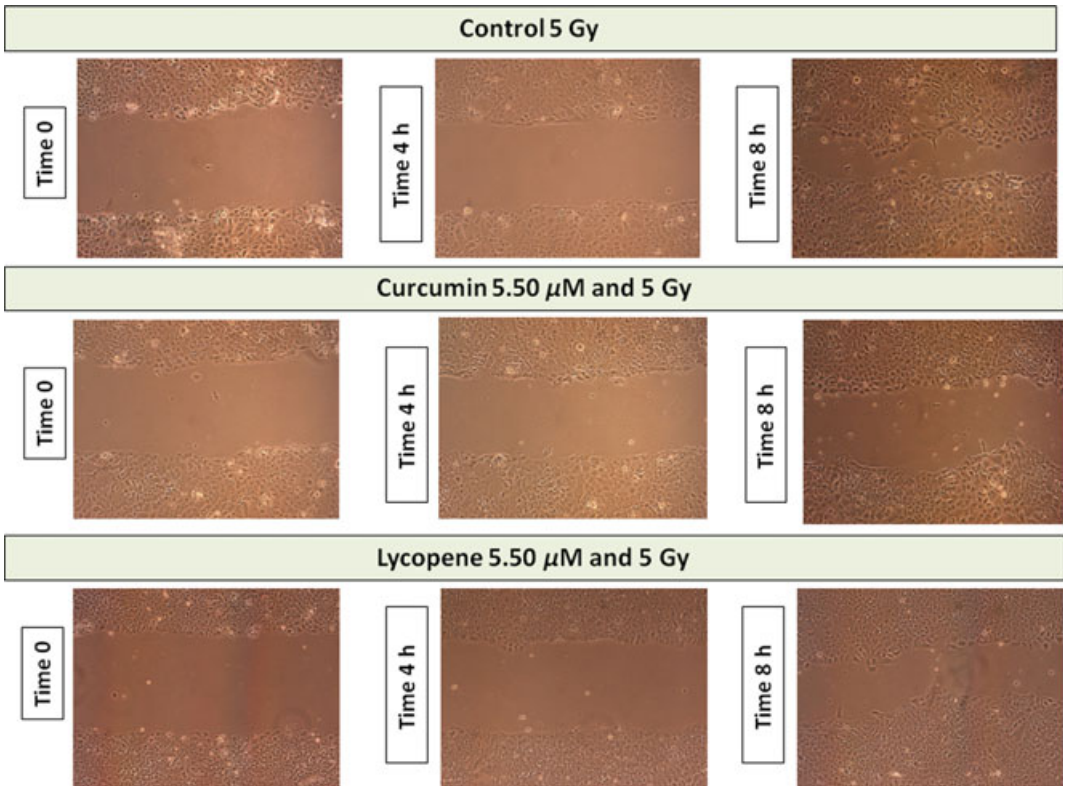

Figure 5 Cell migration into the wound space photographed at the time of the initial wound and at time intervals up to 4 and 8 h postwounding. Results of curcumin and lycopene at a concentration of 5.50 μM and with an irradiation dose of 5 Gy

studies (Beevi *et al*, 2004). Increased levels of free radicals such as malondialdehyde, nitric oxide, and lipid peroxidation and decreased activities of antioxidants including glutathione, ascorbic acid, glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase enzymes have been consistently observed in serum, saliva, and blood leukocytes of patients with oral cancer (Bahar *et al*, 2007; Gokul *et al*, 2010). The increase in ROS and RNS may be the event that leads to the consumption and reduction of antioxidants, explaining the oxidative damage to DNA, proteins, and lipids in oral cancer patients, and possibly the promotion of oral cancer (Gokul *et al*, 2010). Thus, antioxidants should be a necessary part of prevention and therapeutic regimens in oral malignancies. In our study, lycopene exerted a cytotoxic effect upon the PE/CA-PJ15 tumor cells. The magnitude of this effect was directly proportional to the lycopene concentration used – the greatest reduction in cell viability and increase in apoptosis being achieved with doses of 5.50 and 6.75 μM.

Recently, experimental *in vitro* and *in vivo* data have also demonstrated the notorious anticarcinogenic properties of lycopene. In a cell culture study, Livny *et al* (2002) found that lycopene strongly and dose dependently inhibited the proliferation of KB-1 human oral tumor cells, being far more effective in this sense than β-carotene. These results were confirmed by Cheng *et al* (2007), who observed that lycopene suppressed KB cell proliferation at the G0/G1 phase, with a significant decrease in proliferation cell nuclear antigen (PCNA) expression. Cell–cell interaction via gap-junctional communication (GJC) is considered to be a key factor in tissue homeostasis, and its alteration is associated with the neoplastic phenotype. Livny *et al* (Livny *et al*, 2002) showed that lycopene enhanced the gap junction between the KB-1 cells and significantly upregulated the expression of connexin 43, a key protein in the formation of GJC. Taken together, these findings demonstrated that lycopene could suppress the proliferation and promote GJC of oral cancer cells, which may be involved in the mechanisms of action of lycopene in the prevention of oral cancer (Lu *et al*, 2011).

Radiotherapy plays an important role in the management of cancer, contributing to secure local control of tumors following surgery in patients with early stage malignancies. However, radiotherapy alone fails to suppress tumors, which recur and become radioresistant. The factors conditioning such radioresistance in patients with recurring malignancies are not clear. Many studies have reported antioxidants such as vitamin E, curcumin, β-carotene, lycopene, and selenium, in addition to radiotherapy, to have beneficial effects in the management of different types of cancer (HNSCC, prostate, etc.).

Our results indicate a synergic effect against the OSCC cells on combining radiotherapy with curcumin or lycopene treatment. In this sense, such treatment was found to enhance the effect of gamma irradiation on hamster ovarian cells and on the PC-3 human prostate cancer cell line. Using a cell growth and colony-forming (clonogenic) assay, we previously found that curcumin enhances the effect of ionizing irradiation on squamous cell carcinoma

cells *in vitro* (Chendil *et al*, 2004). Cheng *et al* (2001) demonstrated that curcumin, even at high doses (up to 8 g day^{-1}), is non-toxic for patients with premalignant lesions and as such could hypothetically be given for prolonged periods of time (6–7 weeks of irradiation treatments), with minimal side effects. Khafif *et al* (2005) examined whether curcumin can sensitize squamous cell carcinoma cells to the ionizing effects of irradiation. Incubation with curcumin only (3.75 μM) for 48 h did not reduce the number of cells or their ability to form colonies in the absence of irradiation. In plates that were exposed to 1–5 Gy of irradiation, however, the cell counts dropped significantly when pretreated with curcumin – the maximal effect being recorded for the 2.5 Gy irradiation dose. The clonogenic assay revealed a significant decrease in the ability to form colonies following pretreatment with curcumin at all irradiation doses. Likewise, lycopene, whose protective effect against ultraviolet radiation has been well demonstrated by many authors (Fazekas *et al*, 2003; Di Franco *et al*, 2012), also appears to exert a synergic effect when combined with irradiation, in certain types of tumors such as HNSCC or prostate cancer (Tabassum *et al*, 2010).

In the present study, the application of curcumin or lycopene to PE/CA-PJ15 tumor cells during 24, 48, and 72 h of incubation without irradiation exerted an inhibitory effect upon cell viability and increased cell apoptosis. The greatest reduction in cell viability and increase in apoptosis corresponded to the 5.50 and 6.75 μM doses, for both curcumin and lycopene. Likewise, curcumin and lycopene exerted a synergic effect upon both variables when combined with irradiation. Finally, the 5.50 and 6.75 μM doses together with 5 Gy of irradiation induced the greatest reduction in cell migration capacity, with both curcumin and lycopene.

In conclusion, curcumin and lycopene increase cytotoxic activity in the PE/CA-PJ15 cell line and reduce its migratory capacity, while the combination of curcumin or lycopene with irradiation exerts a synergic effect.

Author contributions

FC-A, PL-J and MRT-M were responsible for the study concepts and design; MRT-M was responsible for data acquisition; FC-A was responsible for data analysis. All the authors read and approved the final manuscript.

References

Agarwal S, Rao AV (2000). Tomato lycopene and its roles in human health and chronic diseases. *CMAJ* **163**: 739–744.

Aggarwal BB, Shishodia S (2006). Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem Pharmacol* **71**: 1397–1421.

Aggarwal BB, Kumar A, Bharti AC (2003). Anticancer potential of curcumin preclinical and clinical studies. *Anticancer Res* **23**: 363–398.

Aggarwal BB, Takada Y, Oommen OV (2004a). From chemoprevention to chemotherapy: common targets and common goals. *Expert Opin Investig Drugs* **13**: 1327–1338.

Aggarwal S, Takada Y, Singh S, Myers JN, Aggrawal BB (2004b). Inhibition of growth and survival of human head and neck squamous cell carcinoma cells by curcumin via modulation of nuclear factor-kappaB signalling. *Int J Cancer* **111**: 679–692.

Ammon HPT, Anazodo MI, Safayhi H, Dhawan BN, Srimal RC (1992). Curcumin: a potent inhibitor of Leukotriene B_4 formation in rat peritoneal polymorphonuclear neutrophils (PMNL). *Planta Med* **58**: 226.

Bahar G, Feinmesser R, Shpitzer T, Popovter A, Nagler RM (2007). Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. *Cancer* **109**: 54–59.

Baselga J, Averbuch SD (2000). ZD1839 ('Iressa') as an anticancer agent. *Drugs* **60**: 33–40.

Beevi SS, Rasheed AM, Geetha A (2004). Evaluation of oxidative stress and nitric oxide levels in patients with oral cavity cancer. *Jpn J Clin Oncol* **34**: 379–385.

Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987a). Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res* **47**: 936–942.

Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987b). Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of radiosensitivity. *Cancer Res* **47**: 943–946.

Chakravarti N, Myers JN, Aggarwal BB (2006). Targeting consecutive and interleukin-6-inducible signal transducers and activators of transcription 3 pathway in head and neck squamous cell carcinoma cells by curcumin (diferyloymethane). *Int J Cancer* **119**: 1268–1275.

Chendil D, Ranga RS, Meigooni D, Sathishkumar S, Ahmed MM (2004). Curcumin confers radiosensitizing effect in prostate cancer cell line PC-3. *Oncogene* **23**: 1599–1607.

Cheng AL, Hsu CH, Lin JK *et al* (2001). Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res* **21**: 2895–2900.

Cheng HC, Chein H, Liao CH, Yang YY, Huang SY (2007). Carotenoids suppress proliferating cell nuclear antigen and cyclin D1 expression in oral carcinogenic models. *J Nutr Biochem* **18**: 667–675.

Clark CA, McEachern MD, Shah SH *et al* (2010). Curcumin inhibits carcinogen and nicotine-induced Mammalian target of rampamycin pathway activation in head and neck squamous cell carcinoma. *Cancer Prev Res (Phila)* **3**: 1586–1595.

Clinton SK (1998). Lycopene: chemistry, biology and implications for human health and disease. *Nutr Rev* **56**: 35–51.

Deryugina E, Quigley J (2006). Matrix metalloproteinases and tumor metastasis. *Cancer Metastasis Rev* **25**: 9–34.

Di Franco R, Calvanese M, Murino P *et al* (2012). Skin toxicity from external beam radiation therapy in breast cáncer patients: protective effects of Resveratrol, Lycopene, Vitamin C and anthocianin (Ixor®). *Radiat Oncol* **30**: 12.

Di Mascio P, Kaiser S, Sies H (1989). Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys* **274**: 532–538.

Fazekas Z, Gao D, Saladi RN, Lu Y, Lebwohl M, Wei H (2003). Protective effects of lycopene against ultraviolet B-induced photodamage. *Nutr Cancer* **47**: 181–187.

Gerhartz M, Baranowsky A, Siebolts U *et al* (2007). Morphometric analysis of murine skin wound healing: standardization of experimental procedures and impact of an advanced multitissue array technique. *Wound Repair Regen* **15**: 105–112.

Gokul S, Patil V, Jailkhani R, Hallikeri K, Kattappagari K (2010). Oxidant-antioxidant status in blood and tumor tissue of oral squamous cell carcinoma patients. *Oral Dis* **16**: 29–33.

Gupta PC, Hebert JR, Bhomsle RB, Sinor PN, Mehta H, Mehta FS (1998). Dietary factors in oral leukoplakia and submucous

fibrosis in a population-based case control study in Gujarat, India. *Oral Dis* **4**: 200–206.

Heber D, Lu QY (2002). Overview of mechanisms of action of lycopene. *Exp Biol Med (Maywood)* **227**: 920–923.

Jagetia GC (2007). Radioprotection and radiosensitization by curcumin. *Adv Exp Med Biol* **595**: 301–320.

Johnson JJ, Mukhtar H (2007). Curcumin for chemoprevention of colon cancer. *Cancer Lett* **255**: 170–181.

Khafif A, Hurst R, Kyker K, Fliss DM, Gil Z, Medina JE (2005). Curcumin: a new radio-sensitizer of squamous cell carcinoma cells. *Otolaryngol Head Neck Surg* **132**: 317–321.

Khafif A, Lev-Ari S, Vexler A *et al* (2009). Curcumin: a potential radio-enhancer in head and neck cancer. *Laryngoscope* **119**: 2019–2026.

Kumar A, Bagewadi A, Keluskar V, Singh M (2007). Efficacy of lycopene in the management of oral submucous fibrosis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **103**: 207–213.

Kunjnumakkara A, Anand P, Aggarwal B (2008). Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. *Cancer Lett* **269**: 199–225.

Li L, Braiteh FS, Kurzrock R (2005). Liposome-encapsulated curcumin : in vitro and in vivo effect on proliferation, apoptosis, signaling, and angiogenesis. *Cancer* **104**: 1322–1331.

Livny O, Kaplan I, Reifen R, Polak-Charcon S, Madar Z, Schwartz B (2002). Lycopene inhibits proliferation and enhances gap-junction communication of KB-1 human oral tumor cells. *J Nutr* **132**: 3754–3759.

López-Jornet P, Camacho-Alonso F, Gómez-Garcia F (2011). Effect of curcumin and irradiation in PE/CA-PJ15 oral squamous cell carcinoma. *Acta Odontol Scand* **69**: 269–273.

LoTempio MM, Veena MS, Steele HL *et al* (2005). Curcumin suppresses growth of head and neck squamous cell carcinoma. *Clin Cancer Res* **11**: 6994–7002.

Lu R, Dan H, Wu R *et al* (2011). Lycopene features and potential significance in the oral cancer and precancerous lesions. *J Oral Pathol Med* **40**: 361–368.

Mackerras D, Buffler PA, Randall DE, Nichaman MZ, Pickle LW, Mason TJ (1988). Carotene intake and the risk of laryngeal cancer in coastal Texas. *Am J Epidemiol* **128**: 980–988.

Mayne ST, Cartmel B, Lin H, Zheng T, Goodwin WJ Jr (2004). Low plasma lycopene concentration is associated with increased mortality in a cohort of patients with prior oral, pharynx or larynx cancers. *J Am Coll Nutr* **23**: 34–42.

Moreno-Jiménez M, Valero J, López-Picazo JM *et al* (2010). Concomitant cisplatin, paclitaxel, and hyperfractionated radiotherapy in locally advanced head and neck cancer: comparison of two different schedules. *Am J Clin Oncol* **33**: 137–143.

Nathan C, Amirghahari N, Rice C, Abreo F, Shi R, Stucker F (2002). Molecular analysis of surgical margins in head and neck squamous cell carcinoma patients. *Laryngoscope* **112**: 2129–2140.

Newman DJ, Cragg GM, Snader KM (2003). Natural products as sources of new drugs over the period 1981–2002. *J Nat Prod* **66**: 1022–1037.

O-charoenrat P, Rhys-Evans P, Eccles S (2001). Expression of matrix metaloproteinases and their inhibitors correlates with invasion and metastasis in squamous cell carcinoma of the head and neck. *Arch Otolaryngol Head Neck Surg* **127**: 813–820.

Oridate N, Kim HJ, Xu X, Lotan R (2005). Growth inhibition of heak and neck squamous carcinoma cells by small interfering RNAs targeting eIF4E or cyclin D1 alone or combined with cisplatin. *Cancer Biol Ther* **4**: 318–323.

Parkin DM, Bray F, Ferlay J, Pisani P (2005). Global cancer statistics, 2002. *CA Cancer J Clin* **55**: 74–108.

Petersen PE (2009). Oral cancer prevention and control-the approach of the World Health Organization. *Oral Oncol* **45**: 454–460.

Pignon JP, Bourhis J, Domenge C, Designe L (2000). Chemotherapy added to locoregional treatment for head and neck squamous-cell carcinoma: three meta-analyses of updated individual data MACH-NC Collaborative Group. MetaAnalysis of chemotherapy on head and neck cancer. *Lancet* **355**: 949–955.

Ramachandran C, Rodriguez S, Ramachandran R *et al* (2005). Expression profiles of apoptotic genes induced by curcumin in human breast cancer and mammary epithelial cell lines. *Anticancer Res* **25**: 3293–3302.

Reddy AC, Lokesh BR (1992). Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. *Mol Cell Biochem* **111**: 117–124.

Reid BC, Winn DM, Morse DE, Pendrys DG (2000). Head and neck in situ carcinoma: incidente, trends, and survival. *Oral Oncol* **36**: 414–420.

Reuter S, Eifes S, Dicato M, Aggarwal BB, Diederich M (2008). Modulation of anti-apoptotic and survival pathways by curcumin as a strategy to induce apoptosis in cancer cells. *Biochem Pharmacol* **76**: 1340–1351.

Rogers SJ, Harrington KJ, Rhys-Evans P, O-Charoenrat P, Eccles SA (2005). Biological significance of c-erbB family oncogenes in head and neck cancer. *Cancer Metastasis Rev* **24**: 47–69.

Singh M, Krishanappa R, Bagewadi A, Keluskar V (2004). Efficacy of oral lycopene in the treatment of oral leukoplakia. *Oral Oncol* **40**: 591–596.

Srinivasan M, Rajendra Prasad N, Menon VP (2006). Protective effect of curcumin on gama-radiation induced DNA damage and lipid peroxidation in cultured lymphocytes. *Mutat Res* **611**: 96–103.

Stahl W, Sies H (1996). Lycopene: a biologically important carotenoid for humans? *Arch Biochem Biophys* **336**: 1–9.

Story EN, Kopec RE, Schwartz SJ, Harris GK (2010). An update on the health effects of tomato lycopene. *Annu Rev Food Sci Technol* **1**: 189–210.

Strimpakos AS, Sharma RA (2008). Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials. *Antioxid Redox Signal* **10**: 511–545.

Tabassum A, Bristow RG, Venkateswaran V (2010). Ingestion of selenium and other antioxidants during prostate cancer radiotherapy: a good thing? *Cancer Treat Rev* **36**: 230–234.

Tavani A, Negri E, Franceschi S, Barbone F, La Vecchia C (1994). Attributable risk for laryngeal cancer in Northern Italy. *Cancer Epidemiol Biomark Prev* **3**: 121–125.

Ukai N, Lu Y, Etoh H (1994). Photosensitized oxygenation of lycopene. *Biosci Biotechnol Biochem* **58**: 1718–1719.

Valster A, Tran NL, Nakada M, Berens ME, Chan AY, Symons M (2005). Cell migration and invasion assays. *Methods* **37**: 208–215.

Van Breemen Rb, Pajkovic N (2008). Multitargeted therapy of cancer by lycopene. *Cancer Lett* **269**: 339–351.