

Has Anyone so far Bridged the Gap between microRNA, Botanicals and Oropharyngeal Squamous Cell Carcinoma?

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Abstract

Background/Context: Current debate concerning properties of oropharyngeal squamous cell carcinoma (OSCC) is nothing new. More recent information can be found on functional aspects of microRNA (miRNA) in botanicals and mammals, suggesting that the aging process and cancer development are regulated by these molecules. Interestingly, some dietary supplements (especially botanicals) have been reported to have cancer chemopreventive and/or anti-cancer properties and are common ingredients in so-called "anti-aging" creams.

Objective: The present study was therefore conducted to determine the state-of-the-art on botanicals, OSCC, and their relationship to miRNA.

Methods/Design: PubMed and Google Scholar were interrogated for articles published in English between January 1, 2000 and December 31, 2010 that discuss botanicals and which could possibly be used in treating OSCC. Additional searches in Google, Google Scholar, PubMed and KoreaMed were conducted to detect possible associations between miRNAs and the aforementioned items.

Results: The current body of literature suggests that botanicals might deliver some of their properties via miRNAs. No report was found however that makes a direct connection to OSCC. It therefore has not been determined whether there is an association between botanicals, OSCC, and miRNA molecules that could possibly have "anti-aging" as well as cancer chemopreventive and/or anti-cancer properties.

Conclusions: Systematic studies are needed to determine whether it is miRNA that could dispense possible therapeutic properties of botanicals on OSCC.

Keywords: Botanicals; miRNA; Oropharyngeal Squamous cell carcinoma

Introduction

MicroRNAs (miRNAs) are small post-transcriptional regulators found in animals and plants (Figure 1). Numerous miRNA sequences are evolutionarily conserved among plant species [1,2] as are their target sequences in the mRNAs [3]. This could possibly be due to the role of miRNAs in gene expression control. The average half-life of mammalian miRNAs has been calculated to be 119 hours which suggests that they are up to ten times more stable than mRNAs [4]. Among the differences between plant and animal miRNA biology are the processing, the position of target interaction and/or the degree of complementarity [5]. Health related functions may be mediated by miRNAs which regulate the expressome, metabolome or interactome [6] within plants (esp. botanicals) and/or molecules that modify the status of miRNAs within animals (esp. humans). Tanshinone IIA (Chemical Abstracts Service Registry Number, CAS no. 568-72-9) for

instance, a constituent of the traditional Chinese medicine Dan shen (*Salvia miltiorrhiza*; National Center for Biotechnology Information, NCBI, Taxonomy Identifier: 226208) protects against **particular variants of** sudden cardiac death via repression of miRNA-1 which targets the inwardly rectifying potassium channel subunit Kir2.1 (Universal Protein Resource, UniProt, Identifier: **Q64273**) [7].

Oropharyngeal squamous cell carcinoma (OSCC, figure 2) has been defined as cancer of the anatomical regions that are covered by the classificators C00-C10 [8,9]. It is among the most common cancers worldwide, and continues to represent a public health problem [10]. Despite advances in therapy, cure rates and survival remain poor [11]. OSCC often appears to be preventable and is likely to be related to behavioral and lifestyle factors. Prevention of OSCC therefore remains the goal to reduce the incidence of this disease [12,13].

Anticancerous effects such as growth inhibition of OSCC cell lines can be mediated by miRNAs [14]. It is however noteworthy that some miRNAs can also promote cancer by supporting cell growth or metastasis [15], a phenomenon outside the scope of the present study.

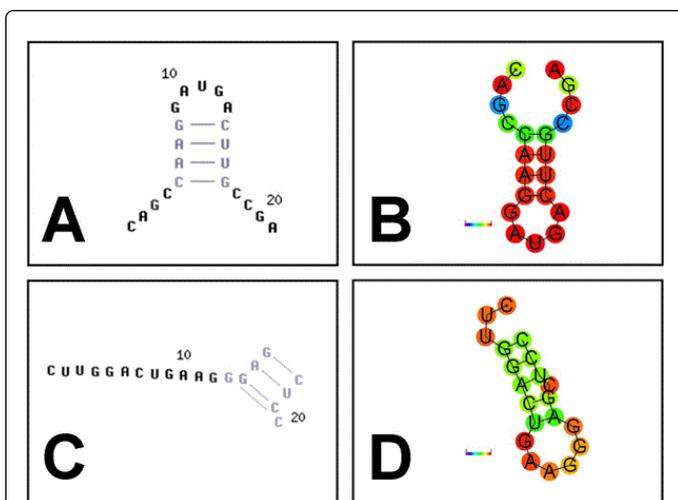


Figure 1: Graphic image representation of miRNA. Exemplarily selected results of single sequence RNA secondary structure prediction experiments for two miRNA species of *Solanum lycopersicum*, sly-miR169c [157] and sly-miR319 [160]. Two different computer programs, CONTRAfold (version: 2.02) [164] and RNAfold (Vienna RNA package version 2.0.0) [165, 166], were used to allow for visual comparison of geometries. [A] A secondary structure suggested for sly-miR169c based on conditional log-linear models (CLLM; CONTRAfold); [B] Coherence was found for the predicted minimum free energy structures and base pair probabilities for sly-miR169c (RNAfold); [C] A CLLM-based secondary structure suggested for sly-miR319 (CONTRAfold); [D] The secondary structure predicted for sly-miR319 based on minimum free energy structures and base pair probabilities (RNAfold) is not coherent, warranting further research

The use of dietary supplements, especially botanicals, or parts of them such as extracts or seeds as medicines predates recorded history and may be seen to represent an antecedent to modern medicine [16]. Technological advancement has facilitated the delivery of breakthrough bioactive compounds of botanicals with potency more or less comparable to those of conventional drugs [17]. Also their low cost, (per) oral bioavailability, and assumed low-risk profile at pharmacologically relevant concentrations have tremendous appeal. As a result of this assumed safety a considerable number of women for example use herbal remedies during pregnancy [18]. Phytochemicals have been evaluated for cancer chemoprevention and anti-cancer activities, increasingly throughout the course of the past decade [19-22]. Interestingly, some of these botanicals are among the top 10 ingredients in 2010 "anti-aging" creams [23]. This and previous reports [24-28] are suggesting that cancer chemopreventive agents and/or anti-aging drugs could have something in common. What they have in common could be effects mediated by miRNA as these molecules play a role in controlling the aging process [29] and cancer development [30]. It may therefore be hypothesized that botanicals could execute the aforementioned cancer chemopreventive and/or anti-cancer effects via miRNAs. The present study was conducted to determine whether miRNAs are currently seen as a link between botanicals and issues related to solid malignant tumors as exemplified by OSCC.

Google [31,32], PubMed [33], Google Scholar [34] and KoreaMed [35] were interrogated to identify scientific literature of relevance to

this topic/area of research. This paper examines what these search engines present when interrogated for articles describing the possible role of miRNA in association with botanicals that have been applied in the context of chemoprevention of OSCC. Information on the role of botanicals in chemoprevention of OSCC published between January 1st, 2000, to December 3rd, 2010, is followed by a summary on miRNAs.

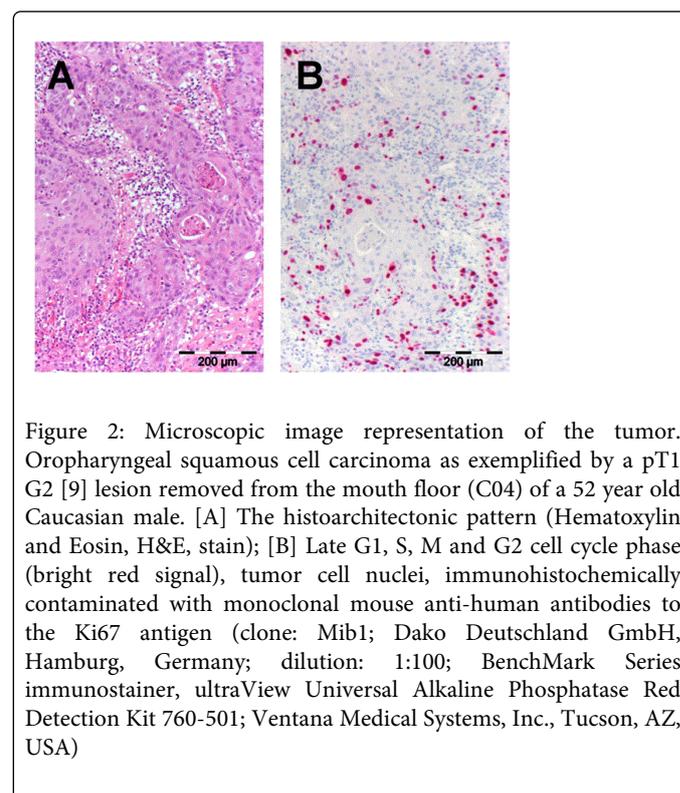


Figure 2: Microscopic image representation of the tumor. Oropharyngeal squamous cell carcinoma as exemplified by a pT1 G2 [9] lesion removed from the mouth floor (C04) of a 52 year old Caucasian male. [A] The histoarchitectonic pattern (Hematoxylin and Eosin, H&E, stain); [B] Late G1, S, M and G2 cell cycle phase (bright red signal), tumor cell nuclei, immunohistochemically contaminated with monoclonal mouse anti-human antibodies to the Ki67 antigen (clone: Mib1; Dako Deutschland GmbH, Hamburg, Germany; dilution: 1:100; BenchMark Series immunostainer, ultraView Universal Alkaline Phosphatase Red Detection Kit 760-501; Ventana Medical Systems, Inc., Tucson, AZ, USA)

Leaves of the trees and shrubs

Olive tree leaves

Olive tree (*Olea europaea* L; NCBI Taxonomy ID: 4146) leaves are well known as a traditional anti-diabetic and anti-hypertensive herbal drug [36]. Extracts rich in oleuropein (CAS no: 32619-42-4, 3 mg/kg body weight; bw) inhibited carcinogenesis in the oropharynx of *Rattus norvegicus* (NCBI Taxonomy ID: 10116), type Fischer (F344), after inductive treatment with 4-Nitroquinoline 1-oxide (4-NQO) (CAS no: 56-57-5) [37].

Neem tree leaves, flowers and fruits

Neem (*Azadirachta indica*; NCBI Taxonomy ID: 124943) is a versatile tree of family Meliaceae (NCBI Taxonomy ID: 43707), the antiproliferative activity of neem tree flowers against some cancer cell lines was established [38]. Ethanolic neem leaf extract (200 mg/kg bw) and neem leaf fractions (1, 10, 100 mg/kg bw, in a dose dependent manner, ddm) have been reported to inhibit the development of OSCC in the buccal pouch of hamsters (NCBI Taxonomy ID: 10036) after application of 7,12-Dimethylbenz(a)anthracene (DMBA) (CAS no: 57-97-6) [39,40]. Apoptosis induction in neem treated hamsters was mediated by the expression of Bim (UniProt: Not Available), Bcl-2 (UniProt: Q687E0), caspase 3 (UniProt: Q60431), and caspase 8

(UniProt: Not Available) [41]. The neem fruit limonoids azadirachtin (CAS no: 11141-17-6; 10, 100 µg/kg bw) and neem flower nimbolide (CAS no: 25990-37-8; 10, 100 µg/kg bw) appeared to block cell proliferative activity and induce apoptosis. Comparatively, nimbolide is a more potent antioxidant and cancer chemopreventive agent [42, 43].

Green, black and yerba mate shrub tea leaves

Tea, from the plant *Camellia sinensis* L. (NCBI Taxonomy ID: 4442), is one of the most common beverages and is generally consumed in its green, black, or oolong form. The main tea extract components are tea polyphenols and tea polysaccharides [44]. Green tea extract (GTE, 0.6% solution in tap water) and curcumin (CAS no: 458-37-7; 10 µmol) [45] had anticancerogenic effects by apoptosis induction, and the inhibition of cell proliferation and angiogenesis. It was proposed that green tea polyphenols (CAS no: 84650-60-2; 200 mg /kg bw/day) may act as a detoxifying agent, altering the expression of glycoconjugates and immunological markers [46], modulating lipid peroxidation and enhancing antioxidant ability [47] in an OSCC model. The recent effect was also associated with black tea polyphenols (BTP, CAS no: 84650-60-2; 0.05% Polyphenon) [47].

Epigallocatechin-3-gallate (CAS no: 989-51-5; 0 to 60 µM or 5, 10, 20, 50 µM, ddm) of green tea decreased the expression of matrix metalloproteinase (MMP) 2 (UniProt: **P08253**) and 9 (UniProt: **P14780**), urokinase plasminogen activator (UPA; UniProt: **P00749**) and inhibited the invasion and migration of carcinoma HSC-2 cells and normal HGF-2 fibroblasts cells [48-50]. A phase II randomized, placebo-controlled trial of GTE (500, 750, or 1,000 mg/m², ddm) concluded that GTE may suppress oropharyngeal premalignant changes, in part by reducing angiogenic stimulation [51].

Black tea was confirmed to have a beneficial effect on oropharyngeal leukoplakia (OLP) [52]. The BTP (30 to 40 mg/kg bw) exerted its preventive effects by inhibiting cell proliferation, modulating the oxidant-antioxidant status as well as markers of cell proliferation, cell survival, tumor infiltration, angiogenesis, apoptosis and xenobiotic-metabolizing enzymes [53-55]. The dietary administration of solitary or combined bovine lactoferrin (UniProt: **Q6LBN7**; 0.2%) and/or BTP (0.05% Polyphenon-B) has been reported to reduce tumor incidence in the hamster buccal pouch (HBP) [56]. Yerba mate tea (236 to 490 mg equiv of chlorogenic/g of dry leaves) is rich in phenolic constituents and can inhibit OSCC proliferation, topoisomerase 1 (UniProt: **P11387**), topoisomerase 2 (UniProt: **P11388**) [57].

Seaside clerodendron shrub leaves

Seaside Clerodendron (*Clerodendrum inerme*; NCBI Taxonomy ID: 49994) is an evergreen sprawling shrub 1-1.8 m tall, containing two sterol constituents [58]. It was found that peroral administration of Clerodendron inerme aqueous leaf extract (500 mg/kg bw) reduced the incidence and size of DMBA-induced OSCC in the HBP [59].

Roots, rhizomes and barks of shrubs herbaceous and trees

Ashwaghandha shrub roots

Ashwaghandha (*Withania somnifera*; NCBI Taxonomy ID: 126910) root extracts are commonly used in Indian traditional Ayurveda as a

general tonic for overall health and remedy for a variety of ailments [60]. Withaferin-A (CAS no: 5119-48-2; 20 mg/kg bw), a steroidal lactone isolated from *Withania somnifera*, appeared to prevent alterations of both p53 (UniProt: **Q00366**) and Bcl-2 and decreases micronucleus formation in the bone marrow once administered to DMBA-painted hamsters [61,62]. Withaferin-A prevented tumor formation and helped maintain erythrocyte integrity during DMBA induction of OSCC [63].

Curcumin and ginger herbaceous plant rhizomes

Curcumin (diferuloylmethane), a polyphenol from the plant *Curcuma longa* (NCBI Taxonomy ID: 136217), traditionally called turmeric is used for cancer chemoprevention and treatment [64]. Meanwhile, ginger (*Zingiber officinale*; NCBI Taxonomy ID: 94328) is one of the plants family members that includes cardamom (NCBI Taxonomy ID: 105181) and turmeric [65]. Curcumin (0.1, 1.0, 10.0 µM, ddm) was considerably more potent than genistein (CAS no: 446-72-0) isoflavone and quercetin (CAS no: 117-39-5) flavonoid, but cisplatin (ATC/DDD: L01XA01; a platinum-based chemotherapy drug; CAS no: 15663-27-1) was fivefold more potent than curcumin in inhibition of growth and DNA synthesis in a cell line (SCC-25) in vitro [66]. In metabolism studies, curcumin (1, 5, 10, 25, and 50 µM, ddm) inhibited P-450 1A1 (UniProt: **P04798**)-mediated by benzo(a)pyrene diol (CAS no: 51689-89-5) bioactivation in both OSCC lines and intact oropharyngeal mucosa [67]. Curcumin (0.1 µM to 1mM) could also induce reactive oxygen species generation and early apoptotic changes in human gingival fibroblasts and human submandibular gland carcinoma cells [68]. It was reported that peroral administration of curcumin (80 mg/kg bw) and piperine (CAS no: 94-62-2; 50 mg/kg bw) to DMBA-painted hamsters helped prevent the formation of OSCC [69].

Moreover, dietary turmeric (1%) augmented apoptosis and decreased cell proliferation in DMBA-treated animals, reflected by decreased tumor burden, multiplicity and enhanced latency period [70]. In highly invasive human YD-10B OSCC cell lines, curcumin inhibited cell proliferation and motility through decreased expression of MMP2 and 9, UPA and UPA receptor (UniProt: Q03405) [71].

Ginger (400 mg powder added to laboratory chow) might have a preventive effect on oropharyngeal carcinogenesis through induction of apoptosis and suppression of tumor growth and proliferation [72].

Portia tree barks

Portia tree or *Thespesia populnea* Soland ex Correa (NCBI Taxonomy ID: 3638; Family: Malvaceae; NCBI Taxonomy ID: 3629) is a large tropical tree found in coastal forests and India [73]. Ethanolic extract of *Thespesia populnea* bark (300 mg/kg bw) has been reported to execute cancer preventive effects in HBP contaminated with DMBA [74].

Fruits and their seeds

Black raspberries

Black raspberries (*Rubus occidentalis*; NCBI Taxonomy ID: 75079) contain many compounds with both in vitro and in vivo preventive properties [75]. The lyophilized black raspberries (5% and 10%) had a preventive activity and inhibited the tumor formation in the oral cavity [76]. Black raspberries inhibitory effects on growth of dysplastic

human cells from the oropharynx were attributed to certain components that target specific signaling pathways regulating the progression of cell cycle [77]. Ethanolic extract (10, 50, 100 µg/ml, ddm) of freeze-dried black raspberries was a promising agent for prevention of oropharyngeal epithelial dysplasia [75]. Application of black raspberry bioadhesive gel (10% w/w gel or 0.5 g of 10% gel) containing anthocyanins (CAS no: 15067-77-7) represented a promising strategy for human OSCC chemoprevention by modulation of gene expression and reduction of cyclooxygenase-2 (UniProt: Q9NNY7) protein [78,79].

Cranberry and grape seeds

Cranberries (*Vaccinium macrocarpon*; NCBI Taxonomy ID: 13750) are a group of **evergreen** dwarf **shrubs** often used by pregnant or breastfeeding women as a preventive agent against urinary tract infections [80]. Grapes (*Vitis vinifera*; NCBI Taxonomy ID: 29760) are considered the world's largest fruit crops, with an approximate annual production of 58 million metric tons [81]. Grape seeds are rich sources of monomeric phenolic compounds, such as catechins (CAS no: 7295-85-4), epicatechin (CAS no: 490-46-0) and epicatechin-3-O-gallate (CAS no: 1257-08-5) and dimeric, trimeric and tetrameric procyanidins (CAS no: 4852-22-6) acting as antimutagenic and antiviral agents [82]. Grape seed proanthocyanidins (CAS no: 84929-27-1) differ from cranberry proanthocyanidins in that they are more likely to contain galloylated flavan-3-ols, and the monomers are primarily B linked [83]. Extracts of grape seed were more cytotoxic than grape peel extracts and 70% methanol extracts of grape seed selectively killed two human oropharyngeal tumor cell lines [84]. Cranberry and grape seed extracts (both 10 to 80 µg/ml, ddm) inhibited the proliferation of OSCC cell lines (CAL27 and SCC25) in a dose-dependent manner [85].

Tomato and its orange colored lycopene

Tomato is an edible fruit of the plant *Solanum lycopersicum* (NCBI Taxonomy ID: 4081) [86]. Combined administration of tomato (0.25 mg lycopene/ml; CAS no: 502-65-8;) and garlic (NCBI Taxonomy ID: 4682; 12.5 mg/ml) inhibited the development of OSCC in the HBP by downregulation of Bcl-2 and upregulation of Bax (UniProt: Not Available), Bim, P53 and the caspases 3 and 8 [87]. They modulated xenobiotic-metabolizing enzymes mitigating the mutagenic and carcinogenic effects of DMBA [88]. Lycopene (a red carotene found in tomatoes; 2.5 mg/kg, bw) might exert its preventive effects by modulating lipid peroxidation and enhancing the activities of the enzymes in the glutathione redox cycle as it reduced glutathione (GSH; CAS no: 70-18-8), glutathione peroxidase (GPx; UniProt: P86215), glutathione S-transferase (GST; UniProt: P30116) and glutathione reductase (UniProt: Not Available) as biomarkers of cancer chemoprevention [89]. Lycopene (3 and 7 mol/L) inhibited proliferation and enhanced gap-junction communication of KB-1 tumor cells from the human oropharynx [90]. The observed effect of lycopene (4 or 8 mg/day) suggested that it can be effectively and safely used for the management of OLP [91]. Tomato paste containing weight lycopene (5 mg/kg bw reduced the incidence of OSCC in the HBP [92].

Flavors and Spices

Garlic and its water soluble extract S-allylcysteine

Garlic (*Allium sativum* L. fam. Alliioideae; Taxonomy ID: 40553) is one of the most commonly used spices and herbal remedies. Alliinase (UniProt: D2CXF2), alliin (sulfur-containing compounds, CAS no: 556-27-4), and allicin (compounds produced enzymatically from alliin, CAS no: 539-86-6) are the main constituents of garlic [93]. Administration of S-allylcysteine (CAS no: 21593-77-1; 200 mg/kg bw) or aqueous garlic extract (250 mg/kg bw) suppressed DMBA-induced oropharyngeal carcinogenesis as revealed by the absence of neoplasms, induction of transglutaminase (UniProt: Not Available) and inhibition of Bcl-2 expression and restored retinoic acid receptor-beta mRNA (UniProt: Q8VHB7) expression to normal [94-97]. S-allylcysteine (0, 2, 5, 10, 20 mM, ddm) effectively inhibited the proliferation, up-regulated the expression of E-cadherin (UniProt: Q9UII7) molecule and stabilized the E-cadherin/beta-catenin (UniProt: P35222) adherent junction complex in human OSCC cell lines [98]. Altered cytokeratin expression and enhanced quantities of circulatory antioxidants were suggested to be associated with garlic-mediated cancer chemoprevention (250 mg/kg bw) of experimental OSCC in the HBP [99,100].

In 4NQO-induced rat tongue carcinogenesis, garlic (25 mg/ml) might execute its preventive effects by modulating lipid peroxidation and enhancing the levels of GSH, GPx (UniProt: P04041) and GST (UniProt: Q6LDP3) [101].

Pepper and its ingredients (piperine and capsaicin)

Piperine (CAS no: 94-62-2) extracted from *Piper longum* (long pepper; NCBI Taxonomy ID: 49511) and *Piper nigrum* (black pepper; NCBI Taxonomy ID: 13216) is an active alkaloid, consumed by a large number of individuals worldwide [102]. Piperine (50 mg/kg bw) had been suggested to execute a chemopreventive effect on DMBA-mediated induction of OSCC in the HBP [103]. The modifying effects of dietary capsaicin (CAS no: 404-86-4; ATC/DDD: N01BX04; active component of chili peppers; NCBI Taxonomy ID: 4073; 500 ppm, w/w) and rotenone (CAS no: 83-79-4; 500 ppm) on initiation or promotion phase of 4-NQO-induced rat tongue carcinogenesis reduced the frequency of OSCC [104].

Plants used in Traditional Chinese Medicine

Levamisole (CAS no: 14769-73-4; ATC/DDD: P02CE01; antihelminthic drug) and/or Chinese medicinal herbs (root of *Astragalus*; NCBI Taxonomy ID: 20400; 12 g/day; fruit of *Ligustrum*; NCBI Taxonomy ID: 104487; 9 g/day; and fruit of *Ziziphi jujuba*; NCBI Taxonomy ID: 157914; 9 g/day) can modulate the level of the serum SCC associated antigen. For OLP patients, the combination therapy was superior to the single therapy of levamisole or of Chinese medicinal herbs [105]. Chinese herbal extracts of *Drynaria bonii* (NCBI Taxonomy ID: 272673), *Angelica sinensis* (NCBI Taxonomy ID: 165353) and *Cornus officinalis* Sieb (NCBI Taxonomy ID: 16906) were investigated for their antitumor potential on human OSCC cell lines (HSC-2 and NA) and the data demonstrated several unique antitumor properties of *Drynaria bonii* [106].

Ching waysan (0, 10, 25, 50, 75 and 100 µl/ml, ddm; table 1) induced apoptosis via a Bax (UniProt: Q07812)-dependent pathway in human OSCC cell lines (OC2 and TSCCa) [107]. Shenyang (1,026 mg

or 256.5 mg/100 g bw) had immune modulatory effects in *rattus norvegicus*, Sprague-Dawley type, with 4-NQO-induced OSCC [108]. Zeng sheng ping (6 g/kg bw/day by gavage) has been reported to prevent OSCC in humans with OLP as well as in animal models [109].

Herba erigerontis (NCBI Taxonomy ID: 124940) appeared to hamper the possible progression of OLP to tumor [110]. Also, Xian huayin (1.7 ml or 11.4 ml/kg bw/day) from China may exhibit a reversal effect on DMBA-induced premalignant lesions in the HBP [111]. The extract of the rhizome of *Coptidium* (NCBI Taxonomy ID: 568508, 13.5% w/w) induced cytochrome-c (UniProt: P99999) dependent apoptosis in immortalized and malignant human oropharyngeal keratinocytes via the mitochondrial signaling pathway [112].

Verticinone (CAS no: 18059-10-4; different concentrations for different cell lines), an alkaloid from *Fritillaria ussuriensis* (NCBI Taxonomy ID: 152096) induced apoptosis through a caspase pathway mediated by mitochondrial damage in immortalized keratinocytes and OSCC cell lines [113]. Rhein (CAS no: 478-43-3) is an anthraquinone (CAS no: 84-65-1) compound from *Rheum palmatum* (NCBI Taxonomy ID: 137221; 0, 25, 50, 100 µM) having inhibitory effects on OSCC as do emodin (CAS no: 518-82-1; 0, 20, 30, 40 µM) and aloemodin (CAS no: 481-72-1; 2.5, 5, 10, 20, 40 µM or 0, 25, 50, 100 µM) [114,115]. Shikonin (CAS no: 517-89-5; 0, 10, 20, 30, 40 µM, ddm), a naphthoquinone (CAS no: 130-15-4) pigment, seemed to alter growth and induce apoptosis in the human OSCC cell line Tca-8113 [116].

Algae and their Extracts

Spirulina platensis

Spirulina (NCBI Taxonomy ID: 1154) is a blue-green filamentous alga (NCBI Taxonomy ID; 1117) [117]. *Spirulina platensis* (*Arthrospira platensis*; NCBI Taxonomy ID: 118562; 10 mg/day) inhibited dysplastic changes occurring in the HBP mucosa and had a long-term cancer regression role on DMBA-induced OSCC [118,119].

Miscellaneous Compounds

Ferulic acid

Ferulic acid (hydroxycinnamic acid, CAS no: 1135-24-6), is found in the brans of grasses such as wheat (*Triticum aestivum*; NCBI Taxonomy ID: 4565), rice (*Oryza sativa*; NCBI Taxonomy ID: 4530), and oats (*Avena*; NCBI Taxonomy ID: 4496) [120]. It (40 mg/kg bw) has been said to reduce tumor incidence and size in DMBA-painted animals by exhibiting antilipidperoxidative effects as well as its ability to modulate the status of carcinogen detoxifying agents [121].

Carnosic acid

Carnosic acid (CAS no: 3650-09-7; 10 mg/kg bw/day) from rosemary (*Rosmarinus officinalis*; NCBI Taxonomy ID: 39367) has been suggested to chemoprevent against DMBA-induced OSCC possibly due to likely carcinogen detoxification and antilipidperoxidative properties [122].

Berberine

Berberine (CAS no: 2086-83-1), an isoquinoline (CAS no: 119-65-3) alkaloid present in roots, rhizome and stem bark of a number of traditional medicinal plants such as *Berberis aquifolium* (NCBI Taxonomy ID: 203270), *Berberis vulgaris* (NCBI Taxonomy ID: 258209), *Berberis aristata* (NCBI Taxonomy ID: 659592) and *Tinospora cordifolia* (NCBI Taxonomy ID: 285590) [123]. The tumor size in xenograft mice treated with berberine (10 mg/kg bw) was smaller than that in the non-treated control group [124]. Prolonged exposure of human HSC-3 OSCC cell lines to berberine (0, 5, 10, 25, 50, 75 µM) increased apoptosis through reduced levels of MMP (UniProt: P03956), release of cytochrome c and activation of caspase 3 (UniProt: P42574) [125]. Moreover, berberine (0, 62.5, 100 µM) was found to down-regulate u-PA, MMP2 and MMP9 expression in SCC-4 cells through the focal adhesion kinase 1 (UniProt: Q05397), inhibitor of nuclear factor (NF) kappa B and NF kappa B mediated pathways and berberine inhibited the invasive capacity of malignant cells [126].

A Few Notes on Dosages

Additional searches were conducted to compare the dosages previously discussed in cell line and animal models with those suggested for humans. The following databases have been interrogated: About Herbs, a web site provided by the Memorial Sloan Kettering Cancer Center (MSKCC Integrative Medicine Service) [127], Herbs at a Glance, and the Complementary and Alternative Medicine (CAM) subset of PubMed both of which have been established by the National Center for Complementary and Alternative Medicine (NCCAM) [128].

This part of the study revealed that the current body of literature contains only few peer reviewed articles on effective clinical dosages in humans specified by good scientific practice experimental design. At least in parts this may be attributable to the fact that botanicals come in a variety of dosage forms such as teas, creams, gels, capsules or tablets, and/or that they can be formulated for various types of drug delivery supporting extended, sustained or other modes of drug release. Different dosages can therefore be readily extracted from the body of literature (table 1). Comparative studies are warranted to learn more about dose-response relationships in this context.

Botanical	Cell Lines	Animal model	Clinical Trial
Tea	Epigallocatechin-3-gallate: 0-60 µM [49] Epigallocatechin-3-gallate: 5-50 µM [50]	Green Tea Extract: 0.6% in tap water [45] Polyphenols: 200 mg/kg bw [46] Polyphenon: 0.05% [47, 56] Black Tea Polyphenon: 30-40 mg/kg bw [54] Yerba Mate Tea: 236-490 mg [57]	Green Tea Extract: 500, 750, 1000 mg/m ² [51] §Catechin: 250 mg/day [167]

Curcumin	Curcumin: 0.1, 1.0 or 10.0 μ M [66]; 1, 5, 10, 25, and 50 μ M [67]; 0.1 μ M to 1mM [68]	Curcumin: 80 mg/kg bw [69] Turmeric: 1% [70]	§Curcumin: 8 g/day [168]; 3.6 g/day [169]; 1-4 g/day [170]
Black Raspberries	Ethanol extract: 10, 50 or 100 μ g/ml [75]	Lyophilized: 5% or 10% [76] Bioadhesive Gel: 1g of 5% gel [78]	Bioadhesive Gel: 0.5g of 10% gel [79]
Tomato/ Lycopene	Lycopene: 3 and 7 mol/L [90]	Lycopene: 0.25 mg/ml [87]; 2.5 mg/kg bw [89]; 5 mg/kg bw [92]	Lycopene: 4 or 8 mg/day [91] §Lycopene: 15 mg/day [171]; 4-30 mg/day [172]
Garlic	S-allylcysteine: 0, 2, 5 10, 20 mM [98]	S-allylcysteine: 200 mg/kg bw [94]; 250 mg/kg bw [96-100]; 25 mg/ml [101]	§Garlic: ~3.5 g/week (range 0 to >28.8 g/week) [173]

Table 1: Dosage Variations. Differences in dosage and pharmaceutical formulation as exemplified above contribute to making systematic and reliable comparative evaluation a considerable challenge. The superscripted section sign ("§") marks entries on lesions other than OSCC

MicroRNA

Medicinal plants may exert the carcinogenic potential by modulating carcinogen detoxification, inhibiting lipid peroxidation, or by improving in vivo antioxidants defense mechanism [90]. Only a few of the described botanicals exhibit articles which describe a role of miRNAs in the corresponding plants. Interrogating PubMed on July 2nd 2011 for entries containing for example the search terms "Salvia miltiorrhiza" and "miRNA" produced only a single hit, a publication mentioning the application of real-time reverse transcription polymerase chain reaction to determine the level of miRNA-1[7]. Entering "Salvia miltiorrhiza" and "miRNA" yielded no findings.

In *Camellia sinensis* there are around 13 conserved miRNAs which belong to 9 miRNA families [129]. Computational homology search in expressed sequencing tags revealed four candidate miRNAs from four miRNA families together with their target [130].

Computational predictions of *Vitis vinifera* genome identified 5.778 putative miRNAs [131]. High-throughput sequencing in *Vitis vinifera* tagged 24 conserved miRNA families, 26 non-conserved miRNA families and 21 grapevine-specific miRNAs [132]. Several grape genes contain putative binding sites of miRNAs [133]. Differential miRNA patterns in tissue development and fruit maturation indicate a function of miRNAs in these processes [134].

In *Solanum lycopersicum* 78 highly conserved mature miRNAs were found by a miRNA detecting microarray [135] and miRNAs play a role in the symbiosis with arbuscular mycorrhizal [136] and draught response (miRNA-169c) [137]. The miRNAs-159[138], 164 [139] and 319 [140] are involved in mechanisms like stamen regulatory network or leaf and flower development of *Solanum lycopersicum*.

In tomato plants the miRNA network is also associated with viral infection and is involved in: host defense response [141], altered host miRNA expression [142] and thereby miRNA mediated gene expression [143].

In safflower at least 236 miRNAs were identified which show different expression patterns in different tissues [144].

High throughput sequencing of wheat small RNAs revealed 58 miRNAs of 43 families from which 4 new miRNAs seem to be specific for monocots [145]. In the Triticeae 28 miRNA precursors were identified together with 337 target sequences in genes [146].

Approximately 40 potential miRNA genes were found in *Oryza sativa* [147]. Altered miRNA expression in rice was found as a result of

heavy metal stress [148] and other biotic and abiotic stress factors [149]. Rice miRNAs control transcription factors which are involved in development and genes that regulate physiological processes [150].

Little is known about botanicals possibly inducing miRNAs in humans. Treatment of carcinoma cells with green tea polyphenons (polyphenon-60 or epigallocatechin gallate) leads to altered expression of miRNAs [151,152]. Epigallocatechin gallate changed miRNA expression in Hep G2 cells [153] and deregulated miRNA-21 and miRNA-330 in a mouse model of prostate carcinoma [154]. In a model of myocardial infarction miRNA-1 was downregulated by Tanshinone IIA a bioactive component of *Salvia miltiorrhiza* [7].

Curcumin up-regulated miRNA-15a and miRNA-16 in MCF-7 breast carcinoma cells and induced apoptosis of A549 human lung carcinoma cells by targeting miRNA-186* [155-157]. In pancreatic carcinoma cell lines miRNA-200 and miRNA-22 were up-regulated and miRNA-21 and miRNA-199* were down-regulated by curcumin (and its analogue CDF) [158,159]. Down-regulation of miRNA-21 by curcumin also inhibited invasion and metastasis in a chicken-embryo-metastasis model [160]. miRNA-146a and IRAK-2 were down-regulated by curcumin in stressed human astroglial cells in primary culture and a mouse model of Alzheimer's disease [161,162].

Extracts of Chinese herbal plant *Coptidis rhizoma* up-regulated miRNA-21 and miRNA-23a in human liver carcinoma cells (MHCC97-L) [163].

For the botanicals, ingredients, and properties that are not listed in this review, because there were no articles found which described the role of miRNAs in the plant or changes in human/animal miRNA expression upon treatment.

Conclusion

Botanical and nutritional compounds have been used for the treatment of cancer throughout human history. So far little is known about the impact of miRNA on expression and function of botanicals. Nevertheless few studies indicate that miRNAs could mediate their health associated processes. Systematic studies are now warranted to determine their role in the chemoprevention of OSCC. To the knowledge of the authors no studies have so far directly bridged the gap between miRNA, botanicals, and the chemoprevention of OSCC. It remains therefore unclear if there is an association between botanicals, OSCC, and miRNA molecules that could possibly have

"anti-aging" as well as cancer chemopreventive and/or anti-cancer properties.

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