Chemical Composition and Antiproliferative Activity of Essential Oil from the Leaves of a Medicinal Herb, *Levisticum officinale*, against UMSCC1 Head and Neck Squamous Carcinoma Cells

SERKAN SERTEL[1,2,3], TOLGA EICHHORN[2,3], PETER K. PLINKERT[1] and THOMAS EFFERTH[2,3]

[1]*Department of Otorhinolaryngology, Head and Neck Surgery, University of Heidelberg, Heidelberg, Germany;*
[2]*Pharmaceutical Biology (C015), German Cancer Research Center, Heidelberg, Germany;*
[3]*Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, University of Mainz, Mainz, Germany*

Abstract. *Background: Oral squamous cell carcinoma (OSCC) is a challenging disease with a high mortality rate. Natural products represent a valuable source for the development of novel anticancer drugs. We investigated the cytotoxic potential of essential oil from the leaves of a medicinal plant, Levisticum officinale (lovage) on head and neck squamous carcinoma cells (HNSCC). Materials and Methods: Cytotoxicity of lovage essential oil was investigated on the HNSCC cell line, UMSCC1. Additionally, we performed pharmacogenomics analyses. Results: Lovage essential oil extract had an IC_{50} value of 292.6 μg/ml. Genes involved in apoptosis, cancer, cellular growth and cell cycle regulation were the most prominently affected in microarray analyses. The three pathways to be most significantly regulated were extracellular signal-regulated kinase 5 (ERK5) signaling, integrin-linked kinase (ILK) signaling, virus entry via endocytic pathways and p53 signaling. Conclusion: Levisticum officinale essential oil inhibits human HNSCC cell growth.*

Oral squamous cell carcinoma (OSCC) is among the top ten most commonly occurring carcinomas worldwide with a high mortality rate. It was estimated that 35,720 people (25,240 men and 10,480 women) were diagnosed with cancer of the oral cavity and pharynx in the United States of America in 2009 and that 7,600 would die of it (1). In spite of advances in therapy, the 5-year survival rate for oral cancer patients has remained at 50% over the past five decades (2). Various multimodal therapy strategies including surgery, radiation and chemotherapy determine the standard treatments for patients with OSCC. However, the treatment of advanced-stage OSCC is associated with morbidity and poor patient outcomes (3). Therefore, alternative therapeutic strategies are called for.

As the majority of anticancer drugs are of natural origin, natural products represent a valuable source for the identification and development of novel treatment options for cancer (4). During the past few decades, research has focused on the health effects of phytochemicals and plant-derived extracts. Plants of the genus *Levisticum* have been attributed with anticancer activity (5, 6). *Levisticum officinale* W. D. J. Koch (lovage) belongs to the family of *Apiaceae*. The name lovage originates from the Latin word *ligusticus* (meaning from Liguria, as the herb used to grow in the Liguria region of northwest Italy).

Apiaceae is a large plant family with about 3,000 species. This family includes many species with medicinal properties which are frequently used in traditional medicine. A common characteristic of this family is the presence of bioactive secondary metabolites in all plant parts: essential oils, polyphenols (flavonoids, phenolic acids), coumarins (furano- and pyranocoumarins), saponins, alkaloids and polyacetylenes (7).

L. officinale has been used as a medicinal plant for centuries due to its carminative, spasmolytic and diuretic effect (8, 9). Clinically it is a potent diuretic (9). It is approved by the German Commission E for use in lower urinary tract infections and urinary gravel (10). The antimycobacterial activity of *L. officinale* is rooted in its polyacetylenes (11).

Correspondence to: Professor Dr. Thomas Efferth, Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, University of Mainz, Staudingerweg 5, 55128 Mainz, Germany. Tel: +49 61313925751, Fax: +49 61313923752, e-mail: efferth@uni-mainz.de

Key Words: Levisticum officinale lovage, oral cavity squamous cell carcinoma OCSCC, head and neck squamous cell carcinoma HNSCC, natural product, pharmacogenomics.

There are no published data concerning the clinical application of *L. officinale* in otorhinolaryngology or experimental data of cytotoxic activity in head and neck squamous cell carcinoma (HNSCC). For this reason, the objective of the present study was to investigate the cytotoxicity of *L. officinale* leaf essential oil towards the human HNSCC cell line, UMSCC1. To gain insight into the molecular mode of action of *Levisticum officinale* leaf essential oil towards the cancer cells, microarray-based mRNA expression profiling was applied. Differentially expressed genes were subjected to signaling pathway analysis.

Materials and Methods

Plant material. *L. officinale* was cultivated in Ross-on-Wye, Herefordshire, UK. Lovage leaf oil was obtained from the leaves by steam distillation (T=100˚C). The procedure was carried out by Norfolk Essential Oils (Norfolk, UK).

The *L. officinale* leaf essential oil used in this study was provided by REHAU AG + Co. (Rehau, Germany).

Cell culture. UMSCC1 cells were originally derived from a male patient with a T2 N0 M0 squamous cell carcinoma of the oral cavity (12). The UMSCC1 cells were cultured in McCoy's medium containing 10% fetal bovine serum (FBS) supplemented with 1% antibiotic-antimycotic (100×), liquid (containing 10,000 units of penicillin (base), 10,000 μg of streptomycin (base) and 25 μg of amphotericin B/ml utilizing penicillin G (sodium salt), streptomycin sulfate and amphotericin B as Fungizone® antimycotic in 0.85% saline) purchased from Invitrogen GmbH (Karlsruhe, Germany). The cells were maintained as monolayers in plastic culture flasks at 37˚C in a humidified atmosphere containing 5% CO_2.

Gas chromatography. The oil extract was analyzed by gas chromatography using an Agilent Technologies 6890N GC instrument and a HP-5 capillary column (0.32 mm × 30 m; 0.25 μm film thickness; Santa Clara, CA, USA). Gas chromatography was performed by Dr. Otto GmbH (Wittenberge, Germany). The oil was diluted with hexane (1:10).

XTT cytotoxicity assay. Cytotoxicity was assessed using the standard 2,3-*bis*[2-methoxy-4-nitro-5-sulfophenyl]-2*H*-tetrazolium-5-carbo-xanilide inner salt (XTT) assay kit (Roche, Indianapolis, USA), which measures the metabolic activity of viable cells (13, 14). The cytotoxicity of *L. officinale* leaf essential oil was determined using the Cell Proliferation Kit II (Roche Diagnostics, Mannheim, Germany). This test is based on the cleavage of the XTT salt by ubiquitous dehydrogenases leading to the formation of an orange formazan dye. The intensity of dye is commensurate to the number of metabolic active cells. A stock solution of *L. officinale* leaf essential oil was prepared in DMSO. A dilution series ranging from 0.54 μg/ml to 18 mg/ml was prepared using DMEM medium to perform the XTT test. The cells were suspended to a final concentration of 1×10^5 cells/ml. 100 μl of the cell suspension were placed into the wells of a 96-well culture plate (Costar, Corning, NY, USA). The marginal wells were filled with 100 μl of McCoy´s medium, in order to minimize evaporation effects. Besides, wells filled with medium were required to determine the background absorbance caused by non-metabolized XTT. A row of wells containing cells was left untreated and another row of wells containing cells was treated with 1 μl DMSO and this served as the solvent control. Each concentration was tested in at least two independent plates containing different batches of cells.

After incubation for 72 h with lovage leaf oil at 37˚C, 5% CO_2 in a humidified atmosphere, XTT reagent was freshly prepared and added to each well as specified by the manufacturer. XTT-labeling reagent and electron-coupling reagent were mixed in a ratio of 50:1 and 50 μl of this mixture were added to each well of the 96-well plate. The plates were incubated for about 3 h at 37˚C, 5% CO_2 in humidified a atmosphere and read out after incubation. Quantification of cell cytotoxicity was performed in an ELISA plate reader (Bio-Rad, München, Germany) at 490 nm with a reference wavelength of 655 nm. The mean absorbance values of the blank wells were subtracted from the experimental values. The cytotoxic effect of the treatment was determined as percentage of viability and compared to untreated cells (13). The toxicity of compounds was determined by means of the formula:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of sample cells}}{\text{Absorbance of untreated cells}} \times 100$$

The simple ligand-binding module of Sigma plot software (version 10.0; Systat, San Jose, CA, USA)) was used for the analysis.

RNA isolation. The total RNA of the UMSCC1 cells was extracted from the test samples using the RNeasy® Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions to obtain highly pure RNA. The isolated total RNA was re-suspended in sample buffer provided by the manufacturer. The concentration and quality of the total RNA were verified by electrophoresis using the total RNA Nanochip assay on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Only the samples with RNA index values greater than 8.5 were selected for expression profiling. RNA concentrations were determined using the NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). All of the RNA samples were stored at –80˚C until used for microarray analyses.

Probe labeling and Illumina Sentrix BeadChip array hybridization. Biotin-labeled cRNA samples for hybridization on Illumina Mouse Sentrix-8 BeadChip arrays (Illumina Inc., San Diego, CA, USA) were prepared according to Illumina's recommended sample labeling procedure based on a previously published protocol (13). In brief, 250 ng total RNA were used for complementary DNA (cDNA) synthesis, followed by an amplification/labeling step (*in vitro* transcription) to synthesize biotin-labeled cRNA according to the MessageAmpII aRNA Amplification kit (Ambion, Inc., Austin, TX, USA). Biotin-16-UTP was purchased from Roche Applied Science, Penzberg, Germany. The cRNA was column purified according to the TotalPrep RNA Amplification Kit, and eluted in 60 μl of water. The quality of cRNA was controlled using the RNA NanoChip Assay on an Agilent 2100 Bioanalyzer and spectrophotometrically quantified (NanoDrop).

Hybridization was performed at 58˚C, in GEX-HCB buffer (Illumina Inc.) at a concentration of 50 ng cRNA/μl in an unsealed wet chamber for 20 h. Spike-in controls for low, medium and highly abundant RNAs were added along with mismatch control and biotinylation control oligonucleotides. The microarrays were

Table I. *Chemical composition of Levisticum officinale leaf essential oil as analyzed by gas chromatography.*

Component	Proportion (%)
α-Terpinyl acetate	48.15
β-Phellandrene	13.16
β-Myrcene	5.07
Ligustilide	3.86
α-Pinene	1.52
α-Phellandrene	0.83
Camphene	0.50
β-Pinene	0.41
Butylidenphthalide (ligusticumlacton)	0.24
Falcarinol	Not detected

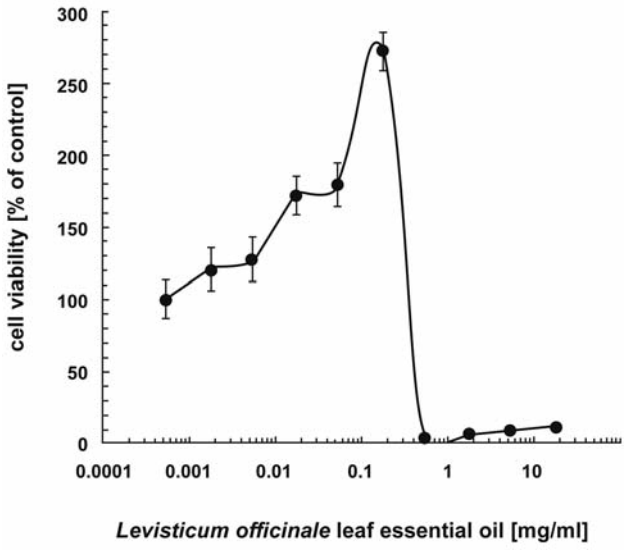

Figure 1. *Cytotoxicity of Levisticum officinale leaf essential oil towards UMSCC1 cells as determined by the XTT assay. Mean dose–response curve of three independent experiments are shown (error bars showing standard error of means).*

washed twice in E1BC buffer (Illumina Inc.) at room temperature for 5 min. After blocking for 5 min in 4 ml of 1% (w/v) blocker casein in phosphate-buffered saline Hammarsten grade (Pierce Biotechnology Inc., Rockford, IL, USA), array signals were developed by 10 min incubation in 2 ml of 1 μg/ml Cy3-streptavidin (Amersham Biosciences, Buckinghamshire, UK) solution and 1% blocking solution. After a final wash in E1BC, the arrays were dried and scanned.

Scanning and data analysis. For microarray scanning, a bead station array scanner (Illumina, Inc.) was adjusted to a scaling factor of 1 and the photomultiplier set to 430. Data extraction was carried out for all the beads individually, and outliers are removed when >2.5 MAD (median absolute deviation). All the remaining data points were used for the calculation of the mean average signal for a given probe, and the standard deviation for each probe was calculated. The RNA from the UMSCC1 cells was subjected to microarray analysis at least twice. The normalized data obtained from the duplicated hybridizations were averaged to obtain a final dataset. Reproducibility of the data was assessed by calculating a percent error (standard deviation/mean ×100) for each gene element.

The data were cropped to a final set of 678 elements by eliminating genes with a differential expression under a single standard deviation. Next, statistical significance was verified by means of empirical Bayes *t*-test and the false discovery rate was corrected with the Benjamini–Hochberg method. Ultimately, genes with $p>0.05$ were discarded after allocation of *p*-values.

Data analysis was carried out with the Chipster analysis software (http://chipster.csc.fi) for DNA microarray expression data by normalization of the signals using the cubic spline algorithm after background subtraction. Differentially regulated genes were defined by calculating the standard deviation differences of a given probe in a one-by-one comparison of samples or groups. For all the genes scored, the fold change and *p*-values were determined.

Signaling pathway analysis. The data obtained from Chipster were analyzed using the Ingenuity Pathways Analysis software (version 6.5) from Ingenuity Systems (Redwood City, CA, USA). This software utilizes the raw image from Chipster in order to align the image and determine expression values for all of the elements.

Statistical analysis. For statistical evaluations, the SPSS 10.0 for Windows software package (SPSS Inc., Chicago, USA) was used. The results were expressed as mean±S.E.M. (standard error of means) of five independent experiments. In the Student's *t*-test, *p*-values <0.05 were considered to be significant.

Results

Essential oil composition. The chemical composition of the oil as assessed by gas chromatography is shown in Table I. *L. officinale* leaf essential oil was characterized by 9 constituents (73.74 % of the total oil). Monoterpenes were the major fraction, of which α-terpinyl acetate was the most abundant compound (48.15 %).

Cytotoxicity. The cytotoxicity of *L. officinale* leaf essential oil towards the human cancer cell line UMSCC1 as determined by the XTT assay is shown in Figure 1. The dose–response curve showed a steady rise in viability to 272.1% compared to the untreated controls at 0.18 mg/ml and a subsequent rapid decrease in viability to 4.7% of control at 0.54 mg/ml. The IC_{50} value calculated from this dose–response curve was IC_{50}=292.6 μg/ml.

Differential gene expression. As determined by microarray hybridization, a total of 678 genes were differentially expressed after treatment with the IC_{50} concentration of lovage leaf oil (292.6 μg/ml). The positive fold changes after log 2-transformation varied in a range of 1.63 to 1.27 and

Table II. *Genes down- or up-regulated after treatment of UMSCC1 cells with Levisticum officinale leaf essential oil.*

Probe ID	Symbol	Description	Fold change [Log_2 ratio]
Down-regulated genes			
4120039	*FTHL2*	Ferritin, heavy polypeptide-like 2	–0.89
1240192	*CSNK1G2*	Casein kinase 1, gamma 2	–0.845
1300072	*SRP14P1*	Signal recognition particle 14 kDa (homologous Alu RNA binding protein) pseudogene 1	–0.8
670181	*LOC85390*	RNA, small nucleolar	–0.715
1470358	*C19orf43*	Chromosome 19 open reading frame 43	–0.71
2370601	*CCDC94*	Coiled-coil domain containing 94	–0.705
4490010	*DAAM1*	Dishevelled-associated activator of morphogenesis 1	–0.69
Up-regulated genes			
4060358	*ABCA1*	ATP-binding cassette, sub-family A (ABC1), member 1	0.705
2070520	*CDCA7*	Cell division cycle associated 7	0.705
7510309	*FRMD6*	FERM domain containing 6	0.69
2970730	*MYADM*	Myeloid-associated differentiation marker	0.69
2940110	*UHRF1*	Ubiquitin-like with PHD and ring finger domains 1	0.69
5810685	*THBS1*	Thrombospondin 1	0.65
6580039	*PKM2*	Pyruvate kinase, muscle	0.64

the negative fold changes varied in a range of 0.53 to 0.78. The seven highest positive (up-regulated genes) and seven lowest negative (down-regulated genes) fold changes are shown in Table II.

Signaling pathway profiling. Ingenuity Pathway Analysis (version 6.5) revealed that out of 64 functional groups of genes listed by the software, 50 were regulated upon lovage essential oil treatment at a significance level of $p<0.05$. The four functional groups of genes (cell death, cancer, cellular growth and proliferation, and cell cycle regulation) with the lowest *p*-values are shown in Figure 2A. Among the 186 signaling pathways analyzed by the pathway analysis software, the four most significantly regulated pathways by lovage leaf oil were the ERK5 signaling, ILK signaling, virus entry *via* endocytic pathways and p53 signaling ($p<0.05$; Figure 2B). The genes associated with these signaling pathways regulated upon lovage leaf oil treatment are shown in Table III.

Discussion

L. officinale ethanol extracts have shown apoptosis-inducing activity in leukemia cell lines (15), but no other cytotoxic activities have been reported. The principle constituents of *L. officinale* have been shown to be polyacetylenes, *i.e.* 3(R)-falcarinol and 3(R)-8(S)-falcarindiol (11). Polyacetylenes of the falcarinol-type have demonstrated many interesting bioactivities including anti-inflammatory (16), antimycobacterial activity (11), antiplatelet-aggregatory (7), cytotoxic (17-19) and antitumor activities (20). 3(R)-8(S)-Falcarindiol was cytotoxic against murine L1210 leukemia cells, only (21). However, in the lovage leaf essential oil, falcarinol was not detected by gas chromatography in the present study. Therefore, we assume that constituents other than falcarinol must have been responsible for the cytotoxic activity in the present investigation. Although α-terpinyl acetate was the major constituent identified, no explicit data concerning its anticancer activity could be found in the literature, but it is reasonable to propose that the cytotoxic activity of *L. officinale* leaf essential oil towards the UMSCC1 cells was mainly due to this constituent. Nevertheless, other constituents of the essential oil may additionally contribute to cytotoxicity.

A surprising and unexpected, but repeatedly observed effect was that subtoxic concentrations of lovage essential oil stimulated proliferation and viability. At higher concentrations, dose-dependent cytotoxic effects were found. Comparable effects have previously been reported for standard anticancer agents such as doxorubicin (22, 23). A proliferation-stimulating effect of otherwise cytotoxic compounds can be interpreted as a rescue mechanism. At low subtoxic concentrations, cancer cells escape detrimental stimuli by the induction of proliferation, while at higher concentrations this defense mechanism is overridden by cytotoxic effects. The possibility that cytotoxic compounds such as doxorubicin or lovage leaf essential oil may exert tumor-promoting activity at low concentrations should also be considered.

The differentially regulated genes after lovage leaf essential oil treatment might help explain the effects on cancer cell growth. *CDCA7* acts as a c-Myc (a transcription factor and major proto-oncogene) responsive gene, and behaves as a direct c-Myc target gene. Overexpression of this gene has been found to enhance the transformation of lymphoblastoid cells, and

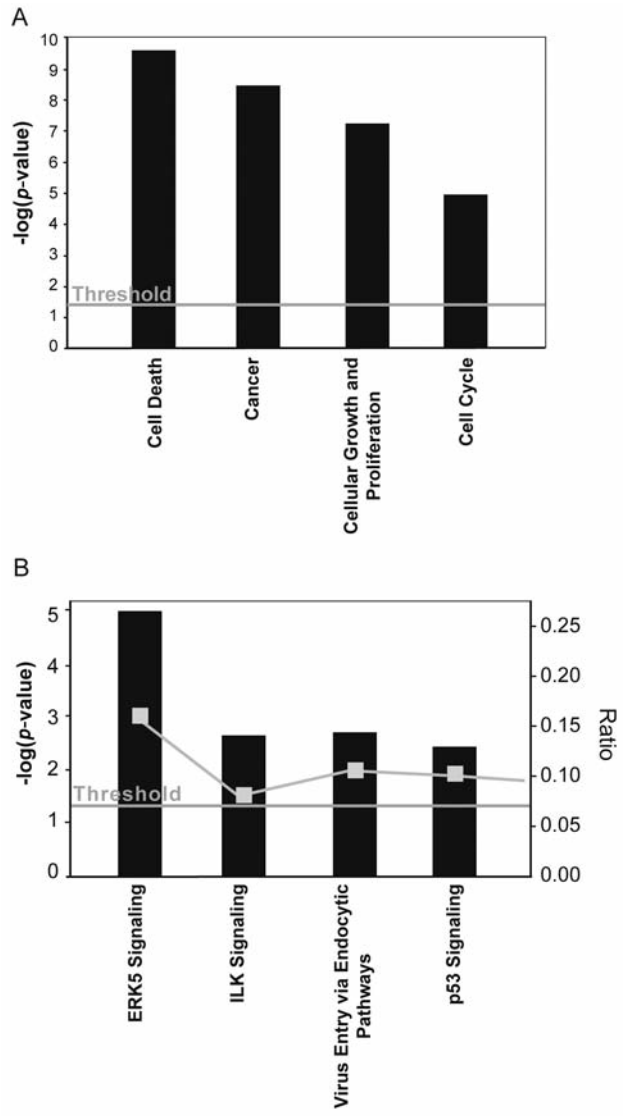

Figure 2. *A: Functional groups of genes and B: Signaling pathways regulated upon Levisticum officinale leaf essential oil treatment in UMSCC1 cells. The evaluation of differentially expressed genes was performed using the Ingenuity Pathway Analysis software.*

contributes to c-Myc-mediated tumorigenesis (24). In the present case, the up-regulated gene *CDCA7* might explain the increase in viable cancer cells at low concentrations of lovage leaf essential oil. *ABCA1* was also an up-regulated gene, encoding a protein belonging to the ATP-binding cassette (ABC) transporters. They are involved in the active transport of phospholipids, ions, peptides, steroids, polysaccharides, amino acids, bile acids, pharmaceutical drugs and other xenobiotic compounds (25). The protein encoded by *ABCA1* is a lipid transporter that plays an important role in cholesterol efflux and thereby prevents toxicity associated with cholesterol overload (24). Gene silencing of *ABCA1*, in resistant M14 melanoma sensitized M14 cells to the apoptotic effect of curcumin. Moreover, *ABCA1* silencing alone also induced apoptosis and reduced p65 expression (26). Thus, it is plausible that up-regulation of *ABCA1* might also explain the increase in viable cancer cells at low concentrations of lovage leaf essential oil. Furthermore, overexpression of *ABCA1* might activate mechanisms of drug resistance and this might contribute to the more aggressive growth of multiple drug-resistant carcinomas.

UHRF1 is another up-regulated gene that might assist the cytotoxicity induced by lovage leaf essential oil treatment. *UHRF1* encodes an ubiquitin ligase that plays a major role in the G_1/S transition and functions in the p53-dependent DNA damage checkpoint, expression of this gene is induced in response to mitogenic stimulation (24, 27).

THBS1 was also an up-regulated gene. *THBS1* encodes adhesive glycoproteins that mediate cell-to-cell and cell-to-matrix interactions. It is a potent inhibitor of tumor growth and angiogenesis (24). These data indicated that the effect of lovage leaf essential oil treatment on the UMSCC1 cells was regulated by multiple genes and that a complex regulatory network contributed to the biological activity. The down-regulated genes did not reveal any association with anticancer activity.

As yet, no data on possible mechanisms of lovage anticancer activity have been reported. Within the present signaling pathway analyses, ERK5 signaling, ILK signaling, virus entry *via* endocytic pathways and p53 signaling were the four most significantly regulated pathways by *L. officinale* essential oil treatment. ERK5, belonging to the MAPK family, is expressed in a variety of tissues and is activated by a range of growth factors, cytokines and cellular stresses. ERK5 signaling is important in endothelial cells for preventing apoptosis, regulating tumor angiogenesis and cell migration (28). The influence of *L. officinale* in ERK5 signaling might offer a novel target for anticancer therapy as an anti-angiogenic agent.

The oncogenic protein kinase, ILK functions as a tumor suppressor protein *in vitro* and *in vivo* in rhabdomyosarcoma (29). It is therefore conceivable that involvement of *L. officinale* might suppress tumorigenesis. No evidence of a link to cancer was found for the virus entry *via* endocytic pathway.

Interestingly, the *L. officinale* essential oil treatment significantly regulated one of the most important tumor suppressor pathways, namely p53 signaling in the UMSCC1 cells. The tumor suppressor protein p53 is activated, *e.g.* upon DNA damage, nucleotide depletion or hypoxia and initiates transcription of pro-apoptotic and cell cycle arrest-inducing target genes (30). Lovage might regulate the activity of p53 by post-translational modifications.

In conclusion, *L. officinale* leaf essential oil has the ability to inhibit tumor cell growth of the HNSCC cell line, UMSCC1, at high concentrations, whereas it promotes proliferation at subtoxic doses. Genes involved in ERK5

Table III. *Signaling pathways with corresponding genes affected by treatment of UMSCC1 with Levisticum officinale leaf essential oil cells (according to http://www.genecards.org).*

Symbol	Description
ERK5 Signaling	
CTF1	Cardiotrophin 1
IL6ST	Interleukin 6 signal transducer (gp130, oncostatin M receptor)
MYC	v-Myc myelocytomatosis viral oncogene homolog (avian)
YWHAE	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide
SGK1	Serum/glucocorticoid regulated kinase 1
CREB3	cAMP-responsive element binding protein 3
WNK1	WNK lysine deficient protein kinase 1
ATF4	Activating transcription factor 4 (tax-responsive enhancer element B67)
MAP3K8	Mitogen-activated protein kinase kinase kinase 8
FOSL1	FOS-like antigen 1
PRKCZ	Protein kinase C, zeta
ILK Signaling	
CREB3	cAMP-responsive element binding protein 3
RAC1	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP-binding protein Rac1)
VIM	Vimentin
MYC	v-Myc myelocytomatosis viral oncogene homolog (avian)
NCK2	NCK adaptor protein 2
MYL9	Myosin, light chain 9, regulatory
RND3	Rho family GTPase 3
IRS1	Insulin receptor substrate 1
RHOA	Ras homolog gene family, member A
ATF4	Activating transcription factor 4 (tax-responsive enhancer element B67)
RSU1	Ras suppressor protein 1
ACTG2	Actin, gamma 2, smooth muscle, enteric
ACTA1	Actin, alpha 1, skeletal muscle
ITGB5	Integrin, beta 5 virus entry *via* endocytic pathways
CD55	CD55 molecule, decay accelerating factor for complement (Cromer blood group)
CAV1	Caveolin 1, caveolae protein, 22 kDa
RAC1	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP-binding protein Rac1)
PLCG1	Phospholipase C, gamma 1
ACTG2	Actin, gamma 2, smooth muscle, enteric
ITGB5	Integrin, beta 5
AP2S1	Adaptor-related protein complex 2, sigma 1 subunit
PRKCZ	Protein kinase C, zeta
ACTA1	Actin, alpha 1, skeletal muscle
p53 Signaling	
TP53INP1	Tumor protein p53 inducible nuclear protein 1
CCND2	Cyclin D2
THBS1	Thrombospondin 1
RAC1	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP-binding protein Rac1)
SERPINB5	Serpin peptidase inhibitor, clade B (ovalbumin), member 5
PMAIP1	Phorbol-12-myristate-13-acetate-induced protein 1
TNFRSF10A	Tumor necrosis factor receptor superfamily, member 10a
CHEK2	CHK2 checkpoint homolog (*S. pombe*)
CHEK1	CHK1 checkpoint homolog (*S. pombe*)

signaling, ILK signaling, virus entry *via* endocytic pathways and p53 signaling are associated with the cytotoxicity of *L. officinale* leaf essential oil.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

Acknowledgements

Thanks are due to Jens-Uwe Bergmann, REHAU AG & Co., for the gift of lovage leaf oil extract.

The UMSCC1 cells were generously donated by Dr. Christian Simon, Director of the Division of Head and Neck Surgical Oncology in the Department of Otorhinolaryngology, Head and Neck Surgery at the University of Heidelberg, Heidelberg, Germany.

References

1 Jemal A, Siegel R, Ward E, Hao Y, Xu J and Thun MJ: Cancer statistics, 2009. CA Cancer J Clin *59*: 225-249, 2009.
2 Chin D, Boyle GM, Porceddu S, Theile DR, Parsons PG and Coman WB: Head and neck cancer: past, present and future. Expert Rev Anticancer Ther *6*: 1111-1118, 2006.
3 Kademani D: Oral cancer. Mayo Clin Proc 82: 878-887, 2007.
4 Newman DJ and Cragg GM: Natural products as sources of new drugs over the last 25 years. J Nat Prod *70*: 461-477, 2007.
5 Xavier CP, Lima CF, Fernandes-Ferreira M and Pereira-Wilson C: *Salvia fruticosa*, *Salvia officinalis*, and rosmarinic acid induce apoptosis and inhibit proliferation of human colorectal cell lines: the role in MAPK/ERK pathway. Nutr Cancer *61*: 564-571, 2009.
6 Loizzo MR, Tundis R, Menichini F, Saab AM and Statti GA: Cytotoxic activity of essential oils from *Labiatae* and *Lauraceae* families against *in vitro* human tumor models. Anticancer Res *27*: 3293-3299, 2007.
7 Christensen LP and Brandt K: Bioactive polyacetylenes in food plants of the *Apiaceae* family: occurrence, bioactivity and analysis. J Pharm Biomed Anal *41*: 683-693, 2006.
8 Segebrecht S and Schilcher H: Ligustilide: guiding component for preparations of *Levisticum officinale* roots. Planta Med *55*: 572-573, 1989.
9 Yarnell E: Botanical medicines for the urinary tract. World J Urol *20*: 285-293, 2002.
10 Blumenthal M, Busse WR and Goldberg A (eds.). The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines. Austin, TX: American Botanical Council; 1998.
11 Schinkovitz A, Stavri M, Gibbons S and Bucar F: Antimycobacterial polyacetylenes from *Levisticum officinale*. Phytother Res *22*: 681-684, 2008.
12 Lin CJ, Grandis JR, Carey TE, Gollin SM, Whiteside TL, Koch WM, Ferris RL and Lai SY: Head and neck squamous cell carcinoma cell lines: established models and rationale for selection. Head Neck *29*: 163-188, 2007.
13 Konkimalla VB, Blunder M, Korn B, Soomro SA, Jansen H, Chang W, Posner GH, Bauer R and Efferth T: Effect of artemisinins and other endoperoxides on nitric oxide-related signaling pathway in RAW 264.7 mouse macrophage cells. Nitric Oxide *19*: 184-191, 2008.
14 Scudiero DA, Shoemaker RH, Paull KD, Monks A, Tierney S, Nofziger TH, Currens MJ, Seniff D and Boyd MR: Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res *48*: 4827-4833, 1988.
15 Bogucka-Kocka A, Smolarz HD and Kocki J: Apoptotic activities of ethanol extracts from some *Apiaceae* on human leukaemia cell lines. Fitoterapia *79*: 487-497, 2008.
16 Metzger BT, Barnes DM and Reed JD: Purple carrot (*Daucus carota* L.) polyacetylenes decrease lipopolysaccharide-induced expression of inflammatory proteins in macrophage and endothelial cells. J Agric Food Chem *56*: 3554-3560, 2008.
17 Matsunaga H, Katano M, Yamamoto H, Mori M and Takata K: Studies on the panaxytriol of *Panax ginseng* C. A. Meyer. Isolation, determination and antitumor activity. Chem Pharm Bull (Tokyo) *37*: 1279-1281, 1989.
18 Matsunaga H, Katano M, Yamamoto H, Fujito H, Mori M and Takata K: Cytotoxic activity of polyacetylene compounds in *Panax ginseng* C.A. Meyer. Chem Pharm Bull (Tokyo) *38*: 3480-3482, 1990.
19 Purup S, Larsen E and Christensen LP: Differential effects of falcarinol and related aliphatic C(17)-polyacetylenes on intestinal cell proliferation. J Agric Food Chem *57*: 8290-8296, 2009.
20 Kobaek-Larsen M, Christensen LP, Vach W, Ritskes-Hoitinga J and Brandt K: Inhibitory effects of feeding with carrots or (–)-falcarinol on development of azoxymethane-induced preneoplastic lesions in the rat colon. J Agric Food Chem *53*: 1823-1827, 2005.
21 Chen QC, Lee J, Jin W, Youn U, Kim H, Lee IS, Zhang X, Song K, Seong Y and Bae K: Cytotoxic constituents from *Angelicae sinensis* radix. Arch Pharm Res *30*: 565-569, 2007.
22 Weisenthal LM, Dill PL, Kurnick NB and Lippman ME: Comparison of dye exclusion assays with a clonogenic assay in the determination of drug-induced cytotoxicity. Cancer Res *43*: 258-264, 1983.
23 Vichi P and Tritton TR: Stimulation of growth in human and murine cells by adriamycin. Cancer Res *49*: 2679-2682, 1989.
24 Genecards. [cited 01.01.2010]; Available from: http://www.genecards.org/.
25 Efferth T: The human ATP-binding cassette transporter genes: from the bench to the bedside. Curr Mol Med *1*: 45-65, 2001.
26 Bachmeier BE, Iancu CM, Killian PH, Kronski E, Mirisola V, Angelini G, Jochum M, Nerlich AG and Pfeffer U: Overexpression of the ATP-binding cassette gene *ABCA1* determines resistance to curcumin in M14 melanoma cells. Mol Cancer *8*: 129-140, 2009.
27 Rebhan M, Chalifa-Caspi V, Prilusky J and Lancet D: GeneCards: a novel functional genomics compendium with automated data mining and query reformulation support. Bioinformatics *14*: 656-664, 1998.
28 Roberts OL, Holmes K, Muller J, Cross DA and Cross MJ: ERK5 and the regulation of endothelial cell function. Biochem Soc Trans *37*: 1254-1259, 2009.
29 Durbin AD, Hannigan GE and Malkin D: Oncogenic ILK, tumor suppression and all that JNK. Cell Cycle *8*: 4060-4066, 2009.
30 Boehme KA and Blattner C: Regulation of p53 – insights into a complex process. Crit Rev Biochem Mol Biol *44*: 367-392, 2009.

Received September 9, 2010
Revised December 6, 2010
Accepted December 7, 2010