The inhibitory mechanism of compound K on A549 lung cancer cells through EGF pathway: an *in silico* and *in vitro* approach

Verónica Castro-Aceituno[1], Muhammad Hanif Siddiqi[2], Sungeun Ahn[1], Natarajan Sathishkumar[2], Hae Yong Noh[1], Shakina Yesmin Simu[2], Zuly E. Jiménez Pérez[2] and Deok Chun Yang[1,2,*]

[1]Department of Oriental Medicinal Biotechnology, and
[2]Graduate School of Biotechnology and Ginseng Bank,
College of Life Sciences, Kyung Hee University,
Yongin, 446-701, Republic of Korea

In previous studies compound K (CK), an active metabolite ginsenoside from *Panax ginseng*, was shown to exhibit anti-cancer activity. However, the mechanism of CK through the EGFR/H-Ras pathway in cancer cells has not been reported so far. Therefore, we focused on the effect of CK as an EGFR and H-Ras inhibitor by *in silico* and *in vitro* studies using A549 cells. The biological activity prediction shows that CK exhibits chemopreventive, anticarcinogenic and antimetastatic activity. Also, using molecular docking studies it has been shown that CK exhibits a strong binding energy with EGFR and H-Ras than Erlotinib. CK inhibits cell viability, decreases cell migration, induces apoptosis and strongly decreases gene expression of EGFR and H-Ras genes *in vitro*. This finding suggests that the EGFR pathway is involved in the anti-cancer activity of CK of EGF-enhanced A549 lung cancer cell line.

Keywords: Epidermal growth factor, lung cancer, molecular docking simulation, *Panax ginseng*.

LUNG cancer is considered the most prevalent form of the disease worldwide. According to the World Health Organization, 1.8 million new cases have been identified in 2012, positioning it as the leading cause of cancer-related mortality worldwide[1]. Around 80–85% of all lung cancer incidences are registered as non-small-cell lung carcinoma (NSCLC)[2]. Overexpression or mutation of epidermal growth factor receptor (EGFR), a cell surface receptor, is related to the development and progression of human malignancies[3–5]. Previous studies reported that EGFR induces cell viability, differentiation and development[6]. The extracellular ligand-binding domain of EGFR interacts with different growth factors such as EGF and TGF-α, which subsequently causes homodimerization or heterodimerization[6]. Due to phosphorylation, tyrosine receptors activate several downstream signalling pathways that stimulate the activation of RAS proto-oncogenes[7–9]. RAS proteins play an important role in lung cancer cell growth, proliferation, apoptosis, disruption in cell cycle, tumour production and human malignancy metastasis[7]. Unfortunately, patients with NSCLC type are diagnosed when the disease has already reached an advanced stage; however, the aim of the treatment is controlling further growth of disease and to give a better life to the patient. Radio and chemotherapy are commonly used for cancer treatment, but the use of these therapies is associated with severe side effects and ultimately decrease in the lifespan of the patient[10]. Thus, the downregulation of EGFR tyrosine kinase might be an effective approach for lung cancer treatment.

Currently, there is considerable interest in the search for EGFR signalling antagonists among natural products[11,12]. The use of natural products in combination with well-known drugs for treatment of human diseases has been reported[13,14]. *Panax ginseng* Meyer is an important medicinal plant in the East Asian countries. Ginsenosides are the major active components of *P. ginseng*[15]. In previous studies, ginsenosides have shown biological activity against several disease[16]. Compound K (CK) is a promising intestinal bacterial metabolite of protopanaxadiol (PPD)-type ginsenosides[17]. Several studies have reported CK as anticancer, antiinflammatory and antioxidant[18–20]. Nevertheless, the EGFR inhibition by CK through the downregulation of EGFR and H-Ras expression has not been reported so far. Therefore, in the present study, we focused on the anti-lung cancer effect of CK as an EGFR inhibitor by *in silico* approach followed by *in vitro* study using A549 lung cancer cells.

Properties such as absorption, distribution, metabolism, excretion and toxicity (ADMET) are important in the discovery of new drugs[21]. Thus we determined the ADMET properties for CK using methods previously described[22]. Also, we predict the possible biological activities of CK based on its chemical structure by PASS Online[23].

From the Protein Data Bank we obtained the three-dimensional structure of the proteins EGFR (PDB ID: 1M17) and H-Ras (PDB ID: 5P21). The 3D structure of H-Ras contains magnesium (Mg), which is an important element for the protein–ligand interactions. Therefore, this element was not removed from the structure. Then, the heteroatom and water molecules were deleted from the structures using Notepad++ (http://notepad-plus-plus.org). Thereafter, the pdbqt files were made using Autodock 4.2.3 software. The Mg charge was added manually in Notepad++. The ligand structure of CK was drawn using ChemSketch program (ACD, Inc., Ontario, Canada) and converted to a 3D structure using Open Babel software[22]. The chemical structure of Erlotinib, a well-known EGFR inhibitor (control for this study), was obtained from PubChem (CID 176870). Finally, the ligand energy was minimized using PyRx 0.8 software

*For correspondence. (e-mail: dcyang@khu.ac.kr)

Table 1. Pharmacokinetic properties of compound K

Properties	CK*	Range of 95% of known drugs
Log BB for brain/blood	–2.384	–3.0/1.2
QP% human oral absorption in GI	55.08	<25% is poor, >80% is high
Aqueous solubility	–5.321	–6.5/0.5
Serum protein binding	0.367	–1.5/1.5
CYP2D6 inhibition	0.306	0 is non-inhibitor and 1 is inhibitor
Hepatotoxicity	0	0 is non-toxic and 1 is toxic
log *P* octanol/water	3.26	–0.2/6.5
Lipinski rule of five violations	2	Maximum 4

(http://pyrxsourceforge.net/downloads) and then saved as .pdbqt format using Autodock 4.2.3.

Autodock Vina 1.1.2 (ref. 24) was used to evaluate the protein–ligand interactions between the proteins EGFR and H-Ras, and the ligands CK and Erlotinib. The active sites Met769 for EGFR, and Thr35, Gly60 and Lys16 for H-Ras were demonstrated to be the most favourable binding sites for docking simulations[22,25]. Next, Discovery Studio 3.5 program (Accelrys Inc., CA, USA) was used for the analysis and the .pdb files and 3D image of the complex structure were prepared. Finally, the 2D structure for EGFR/Erlotinib, EGFR/CK, H-Ras/CK and H-Ras/Erlotinib interactions was generated using LigPlot+ v.1.4.5. (http://ebi.ac.uk).

CK was obtained from the ginseng genetic resource bank (Kyung Hee University, Korea). CK was dissolved in DMSO and stored at –20°C. RPMI-1640 medium with L-glutamine and HEPES (GenDEPOT, USA), FBS (foetal bovine serum) and P/S (penicillin G and streptomycin) (from Gibco-Brl, MD, USA) were acquired. Soluble MTT reagent [3-(4,5-dimethyl-thiazol-2yl)-2,5-diphenyl tetrazolium bromide] (Molecular Probes by Life Technology, OR, USA) and Recombinant Human EGF (236-EG; R&D Systems, MN, USA) were also obtained. Other materials used were commercial products of the highest grade available.

The A549 human lung carcinoma cell line was acquired from the Korean Cell Line Bank, Seoul, South Korea. RPMI-1640 medium supplemented with FBS (10%) and P/S (1%) was used to grow cells at 37°C under 5% CO_2 and 95% humidified atmosphere. MTT reagent was used to measure the cytotoxicity of CK[26]. Briefly, the 96-well culture plate was used to seed the cells at a density of 1×10^5 cells/ml. After incubation overnight, CK treatment was applied to the cells at different concentrations. After 24 h of treatment, 10 µl of MTT (5 mg/ml) was added to each well and incubated for 4 h. Then 100 µl of DMSO was added after removing the old medium and kept for 30 min to dissolve the formazan formed by viable. Absorbance was measured using multimodel plate reader (Bio-Tek Instrument, USA) at a test wavelength of 570 nm with a reference wavelength of 630 nm (ref. 27).

Table 2. Drug-like activity of compound K

Pa	Pi	Biological activity
0.984	0.001	Chemopreventive
0.972	0.001	Anticarcinogenic
0.868	0.005	Antineoplastic
0.777	0.001	Antimetastatic
0.721	0.005	Antineoplastic (lung cancer)
0.419	0.003	Antineoplastic (thyroid cancer)

For wound-healing/scratch assay, A549 cells were seeded in 12-well plate (2.5×10^5 cells/ml) and incubated at 5% CO_2 and 37°C humidified atmosphere. After 80–90% of confluence, the medium was replaced with a fresh one having EGF at 50 ng/ml (EGF-medium). Three hours later, each well was scratched using 10 µl plastic pipette tip and washed with PBS. The medium was replaced with control medium or EGF-medium, following our treatment schedules (2% FBS, 2% FBS + EGF, 2% FBS + 50 µM of CK, 2% FBS+ E GF + 50 µM of CK)[28]. To quantify the scratched region, four randomly selected areas were photographed at × 10 under an optical microscope (Eclipse ME600L, Nikon Instruments, NJ, USA). The images were analysed by Image J program[29].

For gene expression studies, TriZol reagent (Sigma, MO, USA) was used to extract total RNA from A549 cells and retro-transcribed into first-strand cDNAs using cDNA synthesis kit (Thermo Scientific, Lithuania, EU) according to the manufacturer's instructions. Quantitative real-time PCR was performed as described previously[30]. Primer sequences for the markers are as follows: forward EGFR, 5′-CAA CCA AGCT CTC TTG AGG-3′ and reverse 5′-GCT TTC GGA GAT GTT GCT TC-3′; forward RASA1, 5′-TGG GGT CAG AGT TCC TAG GAG-3′ and reverse 5′-AAC GGT ATG GCC ACC TCT T-3; forward β-actin, 5′-ATG AAG TGT GAC GTT GAC ATC C-3′ and reverse 5′-CCT AGA AGC ATT TGC GGT GCA CGA TG-3′. The conditions for amplification were: denaturalization at 95°C for 10 min, followed by 40 cycles at 95°C for 10 s, 60°C for 15 s and 72°C for 20 s.

For fluorescent microscopy/Hoechst 33258 staining assay, A549 cells were seeded in 6-well plate and after

Table 3. Molecular docking results between ligands and proteins

Compound	Target protein	H-bond interaction		Residue	Binding affinity (kcal/mol)	H-bond distance (Å)
		Compound	Macromolecule			
Erlotinib	EGFR	N1	N	MET769	–7.9	3.11
	H-Ras	O4	HZ	LYS16	–8.3	3.17
		O4	HN	GLY13		3.16
Compound K	EGFR	O1	O	MET769	–8.2	3.20
	H-Ras	O6	MG+	THR35	–9.2	2.36
		O5	HZ	LYS16		2.93
		O6	MG+	SER17		2.36
		O6	HN	SER17		3.03
		O8	HN	GLY13		3.22
		O8	HN	GLY13		3.01

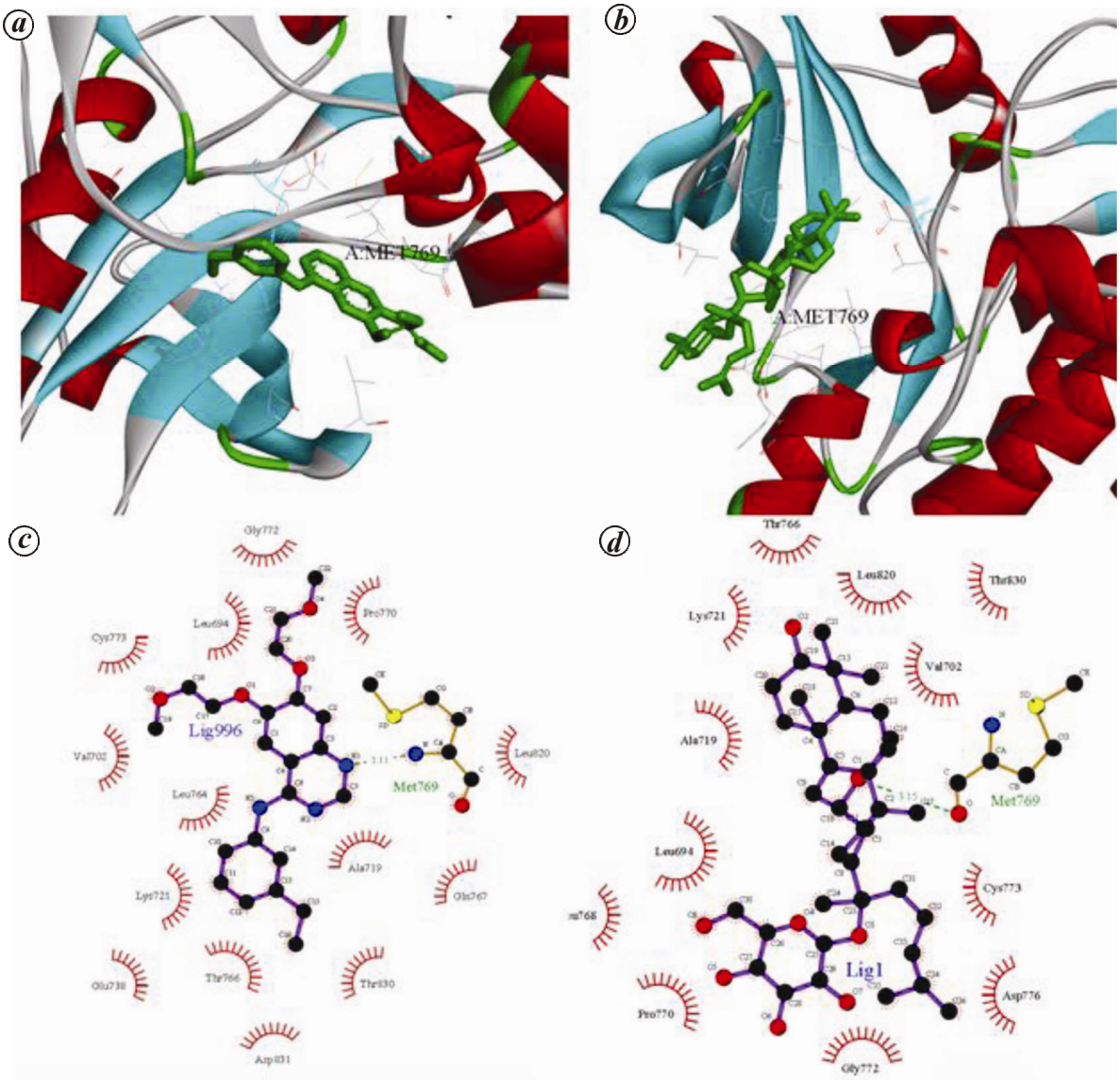

Figure 1. Docking complex structure. The 3D structure of molecular docking results of EGFR between (***a***) Erlotinib and (***b***) compound K (CK). H-bond formation is clearly observed in the 2D structure indicated with arrows, as well as the bond distance (Å) for (***c***) Erlotinib and (***d***) CK.

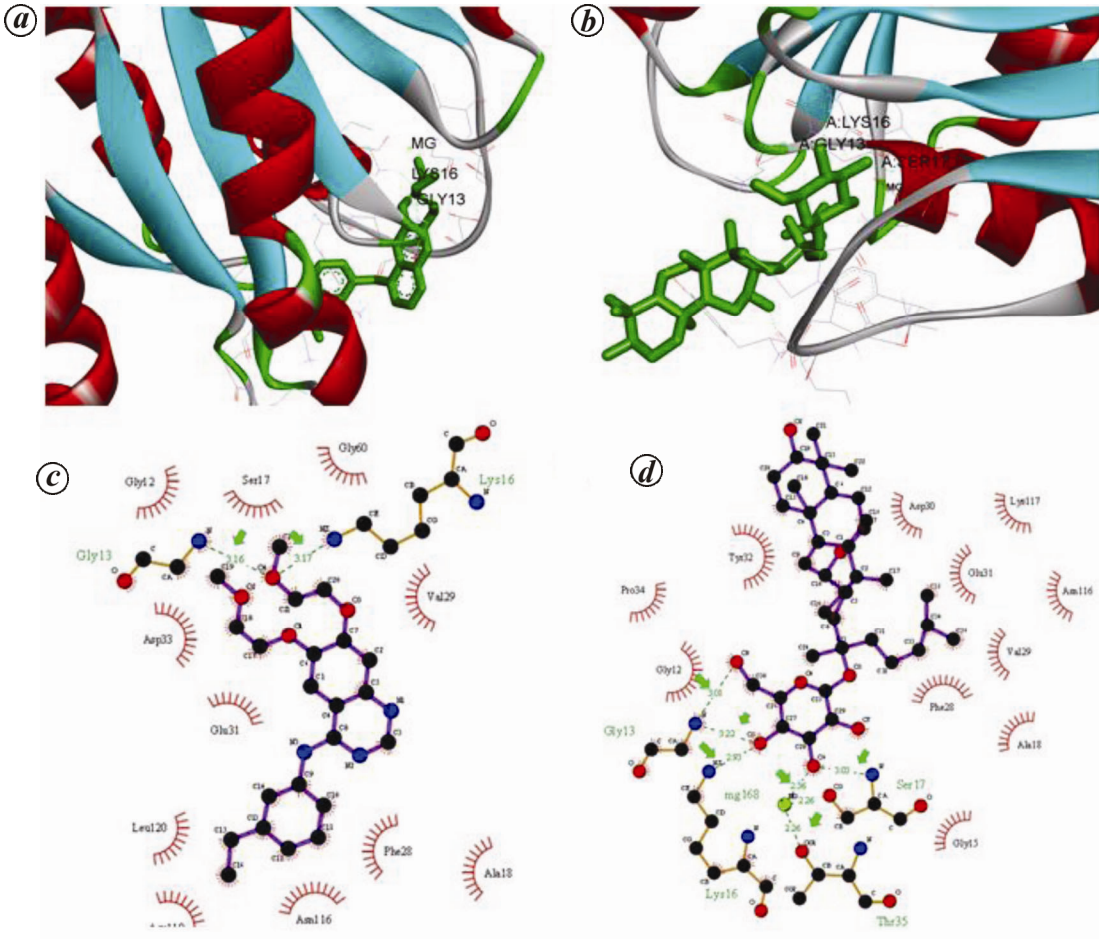

Figure 2. Docking complex structure. The 3D structure of molecular docking results of H-Ras between (***a***) Erlotinib and (***b***) CK. H-bond formation is clearly observed in the 2D structure indicated with arrows, as well as the bond distance (Å) for (***c***) Erlotinib and (***d***) CK.

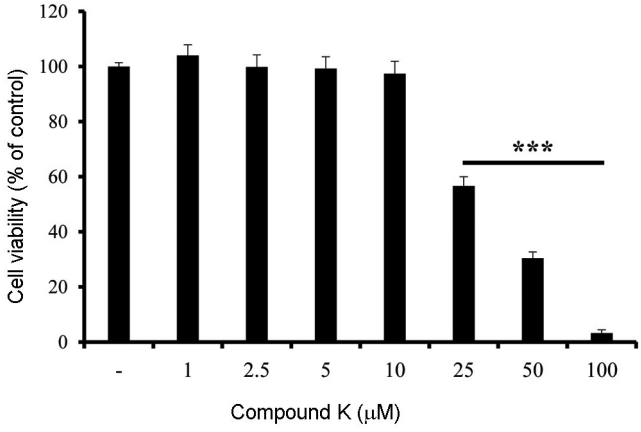

Figure 3. The inhibitory activity of CK on A549 lung cancer cells after 24 h of treatment. Data shown are mean from three independent experiments analysed by ANOVA (***$P < 0.001$ vs control).

24 h, the cells were treated with different concentrations of CK with or without EGF stimulation for 24 h. After washing with 1 ml of 1 × PBS (twice) and fixation with 1 ml of 4% (V/V) formaldehyde (5 min at room temperature), the cells were stained during 20 min with 1 μg/ml Hoechst 33258 (1 ml each well). Stained cells were observed with a fluorescence microscope (Optinity, Korean Labtech).

Statistical significance of differences between values was evaluated by ANOVA. All experiments were repeated more than three times. Values are expressed as mean ± SD; differences are significant at P values <0.05. Analysis was done in Graph Pad 6.04 (La Jolla, CA 92037, USA)[31].

Natural products and their derivatives, alone or in combination with other drugs, are used to increase their potential anticancer activity, which helps reduce their side effects[13,32]. Based on our molecular docking studies for screening the pharmacological activities of *P. ginseng* saponin, we selected a promising ginsenoside such as CK, for the present study[22]. Table 1 shows the pharmacokinetic properties of CK as blood brain barrier (BBB) levels, oral absorption in the gastrointestinal system, hepatotoxicity, aqueous solubility, serum protein binding affinity, CYP2D6 inhibition probability, log P octanol/water and the number or Lipinski rule violations. Table 2

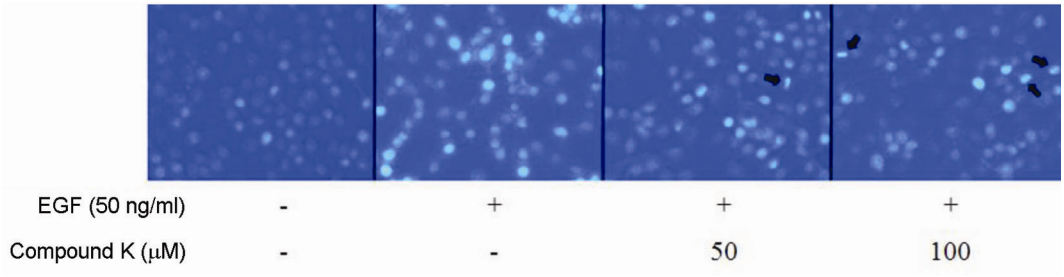

Figure 4. Apoptosis activity of CK on A549 lung cancer cells. Nucleus stained with Hoechst 33258 dye demonstrates the morphological changes. Arrows indicate the apoptotic cell after 24 h of treatment.

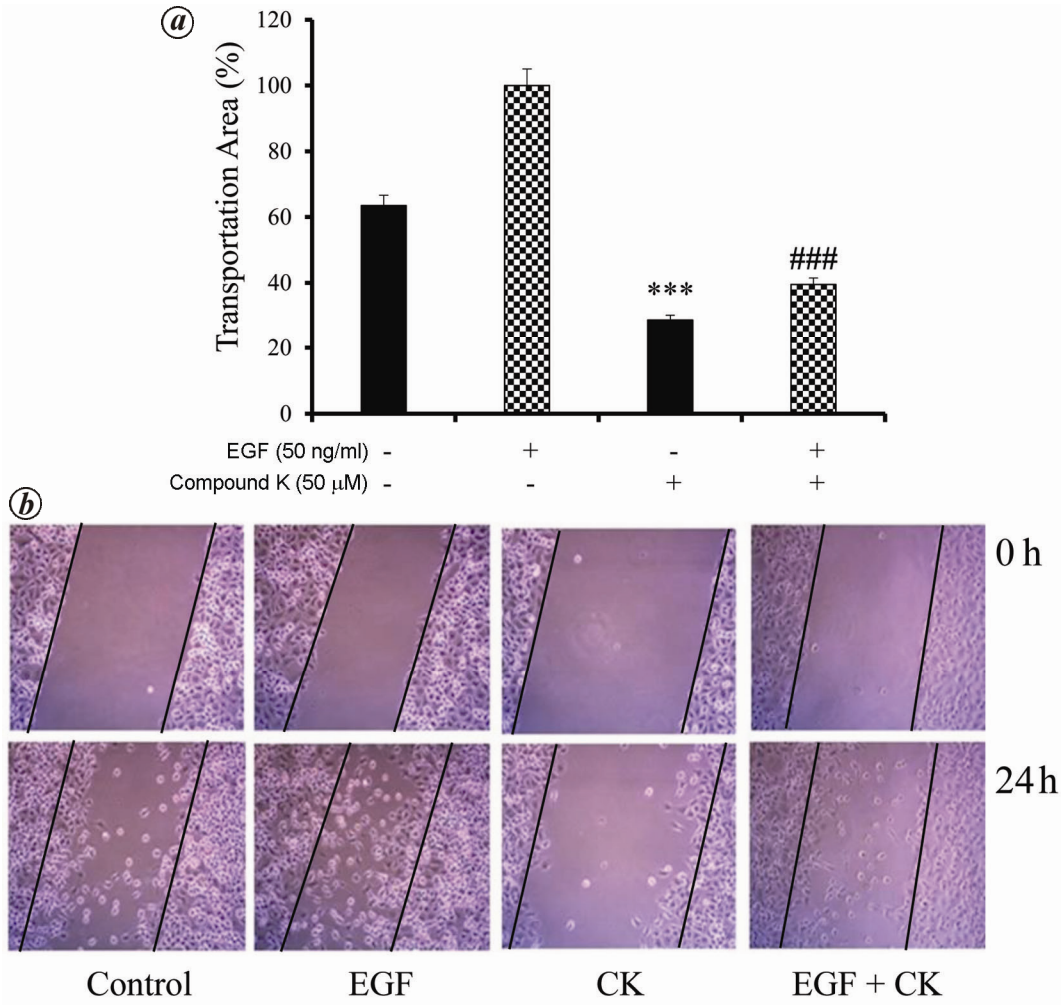

Figure 5. Evaluation of anti-migratory activity of CK on A549 lung cancer cells measured by wound-healing/scratch assay. ***a***, Percentage of cell migration area after 24 h of the scratch. ***b***, Photographs of the cells taken at the moment of the scratch and after 24 h of treatment. Data shown are mean from three independent experiments analysed by ANOVA (***$P < 0.001$ vs control; ###$P < 0.001$ vs EGF).

shows the biological activities of CK such as a chemopreventive, anticarcinogenic, antineoplastic, antimetastatic, antineoplastic (lung cancer) and antineoplastic (thyroid cancer), predicted on the basis of probability of Pa (active) and Pi (inactive) properties using an on-line program to analyse the prediction of activity spectra for substances[23]. According to ADMET studies and PASS analysis, CK could be a candidate for further therapies in the field of cancer treatment according to high selectivity on the activity spectra as an antineoplastic candidate.

Using molecular docking simulation, we analysed the mechanism by which CK might be targeting the EGF-receptor and H-Ras oncoprotein. Our results show that the energy binding used by CK to target EGFR is lower

than Erlotinib (Table 3). Also, CK binds with EGFR through the active site, Met769 (Figure 1). In addition, we analysed the potential of CK to bind with H-Ras, a surface membrane protein, which can be activated after the phosphorylation of EGFR[7]. Similar to our result with EGFR, CK exhibits lower binding energy than Erlotinib (Table 3). CK binds with the active sites Lys16 and Thr35 as well as the active residues Ser17 and Gly13 (Figure 2). According to our findings, we suggest a possible mechanism in which CK not only binds with the surface receptor EGFR, but can also target the surface membrane H-Ras oncoprotein, preventing the activation of this protein by other signalling pathways.

For drug development, the therapeutic effects of new compounds to induce apoptosis in cancer cells can be evaluated by staining the nucleus and observing the morphological change using fluorescent microscopy[33]. Therefore, we examined the apoptotic activity of CK in A549 lung cancer cells. In order to determinate the cytotoxicity of CK, MTT assay was used. The treatment with CK significantly reduced viability of A549 lung cancer cells in a dose-dependent manner (Figure 3). Following this, evaluation of morphological changes in the nucleus by Hoechst 33258 staining was carried out. We found that the number of apoptotic cells after CK treatment had increased by inducing changes in the nucleus such as chromatin condensation (Figure 4). Taken together, we suggest that CK decreases cell viability by induction of apoptosis in a concentration up to 50 μM.

Next the potential antimigratory activity of CK on A549 cells was determinated. A previous study reported that ouabain, a cardioactive glycoside, impedes invasion by decreasing the basal and enhanced migration in human lung cancer A549 cells[28]. Interestingly, it is observed that CK strongly inhibits the motility of lung cancer cells with or without EGF-stimulation (Figure 5), suggesting that this might be an antimigratory activity of CK.

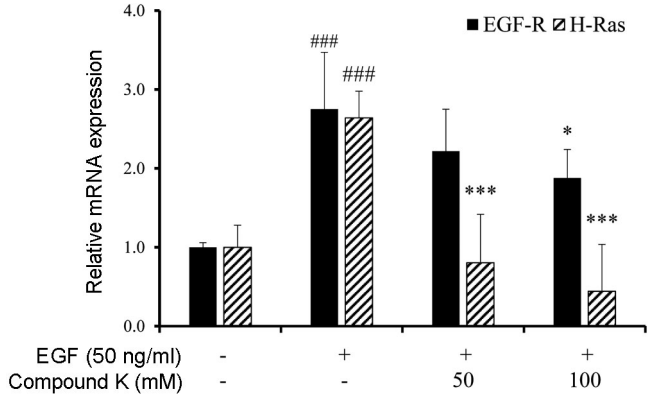

Figure 6. Quantitative gene expression of EGFR and H-Ras in A549 EGF-stimulated lung cancer cells after 24 h of treatment with CK by real-time PCR. Data shown are mean from three independent experiments analysed by ANOVA ($^{###}P < 0.001$ vs control; $^{*}P < 0.05$, $^{***}P < 0.001$ vs EGF-control).

Earlier, it has been reported that upregulation of EGFR plays a critical role in the pathogenesis of lung cancer by stimulating the expression levels of different transcription factors, including H-Ras gene[7–9]. Therefore, to validate the mechanisms of action of CK involved in the inhibition of cell growth, migration and apoptosis effect in A549 cells, we further evaluated the inhibitory effects of CK on mRNA levels of related genes such as EGFR and H-Ras in A549 after stimulation with EGF. Our finding shows that CK suppresses the EGF-induced mRNA expression levels of EGFR and H-Ras genes in A549 cells after 24 h of treatment (Figure 6).

This study suggests that CK exhibits a strong bond with EGFR and H-Ras proteins. Further, treatment of CK can affect cell growth as well as induce apoptosis via the suppression of EGF-stimulated EGFR and H-Ras mRNA expression levels in human lung cancer A549 cells in a dose-dependence manner. This provides a basis for future evaluation of CK and other ginsenosides for the development of new therapeutic agents as anti-lung cancer. However, further *in vivo* study is required for proper understanding of the mechanism of action of CK.

Conflict of interest. The authors have declared that there is no conflict of interest.

1. Ferlay, J. *et al.*, Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer*, 2015, **136**(5), E359–E386.
2. Robinson, C. G. and Bradley, J. D., The treatment of early-stage disease. *Semin. Radiat. Oncol.*, 2010.
3. Franklin, W. A., Veve, R., Hirsch, F. R., Helfrich, B. A. and Bunn, P. A., Epidermal growth factor receptor family in lung cancer and premalignancy. *Semin. Oncol.*, 2002.
4. Harari, P. M. and Huang, S.-M., Epidermal growth factor receptor modulation of radiation response: preclinical and clinical development. *Semin. Radiat. Oncol.*, 2002.
5. Karlin, J. D., Nguyen, D., Yang, S. X. and Lipkowitz, S., Epidermal growth factor receptor expression in breast cancer. *J. Clin. Oncol.*, 2005, **23**(31), 8118–8119.
6. Janmaat, M. and Giaccone, G., The epidermal growth factor receptor pathway and its inhibition as anticancer therapy. *Drugs Today*, 2003, **39**, 61–80.
7. Danesi, R., De Braud, F., Fogli, S., De Pas, T. M., Di Paolo, A., Curigliano, G. and Del Tacca, M., Pharmacogenetics of anticancer drug sensitivity in non-small cell lung cancer. *Pharmacol. Rev.*, 2003, **55**(1), 57–103.
8. Schlessinger, J., Cell signaling by receptor tyrosine kinases. *Cell*, 2000, **103**(2), 211–225.
9. Woodburn, J., The epidermal growth factor receptor and its inhibition in cancer therapy. *Pharmacol. Ther.*, 1999, **82**(2), 241–250.
10. Group NM-AC, Chemotherapy in addition to supportive care improves survival in advanced non-small-cell lung cancer: a systematic review and meta-analysis of individual patient data from 16 randomized controlled trials. *J. Clin. Oncol.*, 2008, **26**(28), 4617.
11. Madhusudan, S. and Ganesan, T. S., Tyrosine kinase inhibitors in cancer therapy. *Clin. Biochem.*, 2004, **37**(7), 618–635.

12. Shawver, L. K., Slamon, D. and Ullrich, A., Smart drugs: tyrosine kinase inhibitors in cancer therapy. *Cancer Cell*, 2002, **1**(2), 117–123.
13. Leung, K. W. and Wong, A. S., Pharmacology of ginsenosides: a literature review. *Chin. Med.*, 2010, **5**(1), 20.
14. Shin, H. R., Kim, J. Y., Yun, T. K., Morgan, G. and Vainio, H., The cancer-preventive potential of *Panax ginseng*: a review of human and experimental evidence. *Cancer Causes Control*, 2000, **11**(6), 565–576.
15. Park, J. D., Rhee, D. K. and Lee, Y. H., Biological activities and chemistry of saponins from *Panax ginseng* CA Meyer. *Phytochem. Rev.*, 2005, **4**(2–3), 159–175.
16. Kim, B. J., Involvement of melastatin type transient receptor potential 7 channels in ginsenoside Rd-induced apoptosis in gastric and breast cancer cells. *J. Ginseng Res.*, 2013, **37**(2), 201.
17. Tawab, M. A., Bahr, U., Karas, M., Wurglics, M. and Schubert-Zsilavecz, M., Degradation of ginsenosides in humans after oral administration. *Drug Metab. Dispos.*, 2003, **31**(8), 1065–1071.
18. Jeong, A., Lee, H.-J., Jeong, S.-J., Lee, H.-J., Lee, E.-O., Bae, H. and Kim, S.-H., Compound K inhibits basic fibroblast growth factor-induced angiogenesis via regulation of p38 mitogen activated protein Kinaseand AKT in human umbilical vein endothelial cells. *Biol. Pharm. Bull.*, 2010, **33**(6), 945–950.
19. Lee, I. K. *et al.*, Compound K, a metabolite of ginseng saponin, induces mitochondria-dependent and caspase-dependent apoptosis via the generation of reactive oxygen species in human colon cancer cells. *Int. J. Mol. Sci.*, 2010, **11**(12), 4916–4931.
20. Mathiyalagan, R., Subramaniyam, S., Kim, Y. J., Natarajan, S., Min, J. W., Kim, S. Y. and Yang, D. C., Synthesis and pharmacokinetic characterization of a pH-sensitive polyethylene glycol ginsenoside CK (PEG-CK) conjugate. *Biosci. Biotechnol. Biochem.*, 2014, **78**(3), 466–468.
21. Benet, L. Z., Kroetz, D., Sheiner, L., Hardman, J. and Limbird, L., Pharmacokinetics: the dynamics of drug absorption, distribution, metabolism, and elimination. *Goodman and Gilman's the Pharmacological Basis of Therapeutics*, 1996, pp. 3–27.
22. Sathishkumar, N., Karpagam, V., Sathiyamoorthy, S., Woo, M. J., Kim, Y.-J. and Yang, D.-C., Computer-aided identification of EGFR tyrosine kinase inhibitors using ginsenosides from *Panax ginseng*. *Comput. Biol. Med.*, 2013, **43**(6), 786–797.
23. Poroikov, V. V. *et al.*, PASS biological activity spectrum predictions in the enhanced open NCI database browser. *J. Chem. Inf. Comput. Sci.*, 2003, **43**(1), 228–236.
24. Trott, O. and Olson, A. J., AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.*, 2010, **31**([Trott, 2010 #35]), 455–461.
25. Pai, E. F., Krengel, U., Petsko, G. A., Goody, R. S., Kabsch, W. and Wittinghofer, A., Refined crystal structure of the triphosphate conformation of H-Ras p21 at 1.35 A resolution: implications for the mechanism of GTP hydrolysis. *EMBO J.*, 1990, **9**(8), 2351.
26. Ranga, R. S., Sowmyalakshmi, S., Burikhanov, R., Akbarsha, M. A. and Chendil, D., A herbal medicine for the treatment of lung cancer. *Mol. Cell Biochem.*, 2005, **280**(1–2), 125–133.
27. Aceituno, V. C., Ahn, S., Simu, S. Y., Wang, C., Mathiyalagan, R. and Yang, D. C., Silver nanoparticles from Dendropanax morbifera Léveille inhibit cell migration, induce apoptosis, and increase generation of reactive oxygen species in A549 lung cancer cells. *In Vitro Cell. Develop. Biol.-Anim.*, 2016, 1–8.
28. Liu, N., Li, Y., Su, S., Wang, N., Wang, H. and Li, J., Inhibition of cell migration by ouabain in the A549 human lung cancer cell line. *Oncol. Lett.*, 2013, **6**(2), 475–479.
29. Collins, T. J., ImageJ for microscopy. *BioTechniques*, 2007, **43**(1 Suppl), 25–30.
30. Siddiqi, M. H., Siddiqi, M. Z., Ahn, S., Kim, Y.-J. and Yang, D. C., Ginsenoside Rh1 induces mouse osteoblast growth and differentiation through the bone morphogenetic protein 2/runt-related gene 2 signalling pathway. *J. Pharm. Pharmacol.*, 2014, **66**(12), 1763–1773.
31. Siraj, F. M., Natarajan, S., Huq, M. A., Kim, Y. J. and Yang, D. C., Structural investigation of ginsenoside Rf with PPARγ major transcriptional factor of adipogenesis and its impact on adipocyte. *J. Ginseng Res.*, 2014.
32. Choi, K.-T., Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax ginseng* CA Meyer. *Acta Pharmacol. Sin*, 2008, **29**(9), 1109.
33. Hwang, J. W. *et al.*, Mountain ginseng extract exhibits anti-lung cancer activity by inhibiting the nuclear translocation of NF-κB. *Am. J. Chin. Med.*, 2012, **40**(01), 187–202.

ACKNOWLEDGEMENTS. This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries, Republic of Korea (313038-03-2-SB010).

Received 15 July 2015; revised accepted 19 April 2016

doi: 10.18520/cs/v111/i6/1071-1077

Microbial water desalination and bio-electricity generation – role of biomass carbon

A. Carmalin Sophia* and V. M. Bhalambaal

National Environmental Engineering Research Institute, CSIR, Taramani, Chennai 600 113, India

Microbial desalination cells (MDCs) are modified microbial fuel cells (MFC) that are energy-sustainable. They use organic matter in wastewater as the energy source for desalination. The electric potential gradient is caused by exoelectrogenic bacteria. A typical MDC has a middle compartment for water desalination between the anode and cathode chambers. Our study reports lab-scale desalination, for evaluating the role of carbon from biomass waste, i.e. coconut shells. Control experiments were performed in the absence of activated carbon. Different initial salt concentrations (25 and 35 gl^{-1}) were investigated. MDC produced a maximum voltage of 460 ± 13 mV simultaneously removing about 83.3 ± 1.3% of Na^+ and 57.8 ± 1.1% of Cl^-, in the desalination cycle. The control MDC produced a maximum of 260 ± 8 mV and 69.3 ± 2% of Na^+ removal and 51 ± 1.5% Cl^- removal. These results explain the role of using activated carbon for improved power production and water desalination.

*For correspondence. (e-mail: ac_sophia@neeri.res.in)