Biochemical and Biophysical Research Communications 398 (2010) 74–78

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The dietary flavonoid kaempferol effectively inhibits HIF-1 activity and hepatoma cancer cell viability under hypoxic conditions

Ilias Mylonis, Achillia Lakka, Andreas Tsakalof, George Simos *

Laboratory of Biochemistry, School of Medicine, University of Thessaly, BIOPOLIS, 41110 Larissa, Greece
Institute of Biomedical Research and Technology (BIOMED), 51 Papanastasiou str., 41222 Larissa, Greece

ARTICLE INFO

Article history:
Received 8 June 2010
Available online 15 June 2010

Keywords:
Hypoxia
Hypoxia-inducible factor 1
HIF-1α
MAPK
Huh7
Hepatocellular carcinoma
Flavonoids
Kaempferol

ABSTRACT

Hepatocellular carcinoma (HCC) is characterized by high mortality rates and resistance to conventional treatment. HCC tumors usually develop local hypoxia, which stimulates proliferation of cancer cells and renders them resilient to chemotherapy. Adaptation of tumor cells to the hypoxic conditions depends on the hypoxia-inducible factor 1 (HIF-1). Over-expression of its regulated HIF-1α subunit, an important target of anti-cancer therapy, is observed in many cancers including HCC and is associated with severity of tumor growth and poor patient prognosis. In this report we investigate the effect of the dietary flavonoid kaempferol on activity, expression levels and localization of HIF-1α as well as viability of human hepatoma (Huh7) cancer cells. Treatment of Huh7 cells with kaempferol under hypoxic conditions (1% oxygen) effectively inhibited HIF-1 activity in a dose-dependent manner (IC_{50} = 5.16 μM). The mechanism of this inhibition did not involve suppression of HIF-1α protein levels but rather its mislocalization into the cytoplasm due to inactivation of p44/42 MAPK by kaempferol (IC_{50} = 4.75 μM). Exposure of Huh7 cells to 10 μM kaempferol caused significant reduction of their viability, which was remarkably more evident under hypoxic conditions. In conclusion, kaempferol, a non-toxic natural food component, inhibits both MAPK and HIF-1 activity at physiologically relevant concentrations (5–10 μM) and suppresses hepatocarcinoma cell survival more efficiently under hypoxia. It has, therefore, potential as a therapeutic or chemopreventive anti-HCC agent.

1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and is associated with high mortality. It is characterized by intrinsic drug-metabolizing activity that confers resistance to chemotherapeutic treatment and, until now, therapies depend on patient and cancer stage. Early detection allows HCC treatment by transplantation, surgical resection or local ablation, whereas at an intermediate stage transarterial chemoembolization can be applied in some cases [1]. Important factors of HCC development are, among others, angiogenesis and signaling cascades regulating cell proliferation such as the Raf/MEK/MAPK pathway, which is activated in many hepatocarcinoma-derived cell lines and tumor samples [2,3]. Current drug development aims at the inhibition of the MAPK pathway as exemplified by sorafenib, a potent Raf kinase inhibitor [4], which causes tumor regression in HCC models and prolongation of survival in HCC patients [5].

Another aspect of HCC, like in many other human solid tumors, is the development of hypoxic regions due to increased cell proliferation and limited blood supply. Hypoxia can further promote HCC progression by facilitating angiogenesis and metabolic adaptation [6]. A key component of adaptation to oxygen deprivation is the hypoxia-inducible factor-1 (HIF-1), a transcriptional activator of cell survival, proliferation, angiogenesis, invasion and metastasis genes [7]. Its regulated alpha subunit (HIF-1α) represents an attractive therapeutic target because of its expression in many cancers and its association with poor patient prognosis [8]. Expression of HIF-1α in HCC has been associated with VEGF secretion and malignant transformation [9–11].

We have recently shown that p44/42 MAPK targets the C-terminal domain of HIF-1α and promotes its nuclear accumulation and activity by masking a CRM1-dependent nuclear export signal [12,13]. We have also reported that several flavonoids, natural occurring dietary polyphenolic compounds, can inactivate p44/42 MAPK and cause inhibition of HIF-1α phosphorylation and activity [14]. Given the importance of the MAPK pathway as well as HIF-1 for the growth of HCC, we now report the effect of the flavonoid

Abbreviations: HCC, hepatocellular carcinoma; HIF-1, hypoxia inducible factor-1; MAPK, mitogen activated protein kinase; DMSO, dimethyl sulfoxide.

* Corresponding author at: Laboratory of Biochemistry, School of Medicine, University of Thessaly, BIOPOLIS 41110, Larissa, Greece. Fax: +30 2410 685545.
E-mail address: simos@med.uth.gr (G. Simos).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.06.038

kaempferol on HIF-1 activity and survival of hepatocarcinoma-derived Huh7 cells incubated under hypoxia, a condition that simulates growth inside a solid tumor.

2. Materials and methods

2.1. Cell culture and reporter gene assays

Huh7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) and 100 U/ml penicillin–streptomycin (Gibco, CA, USA). Transfections with the hypoxia-responsive reporter pGL3–5HRE-VEGF and the *Renilla* luciferase-expressing plasmids were performed as described previously [12]. When required, cells were treated for 4 h with kaempferol (1–100 μM, Sigma, UK) using a 10 mM stock solution in dimethyl sulfoxide (DMSO). For hypoxic treatment, cells were exposed for 4 h to 1 mM dimethyloxalyl glycine (DMOG; Alexis Biochemicals) or for 4–48 h to 1% O_2, 95% N_2 and 5% CO_2 in an IN VIVO$_2$ 200 hypoxia workstation (RUSKINN Life Sciences, UK). When required, hypoxic conditions were adjusted to 0.1% O_2, 95.9% N_2 and 5% CO_2. For reporter gene assays, cells were washed with PBS and lysed 24 h post-transfection and luciferase activity was determined using the luciferase assay kit (Promega, WI, USA). Firefly luciferase activity was normalized to *Renilla* activity and values are presented as the means of three independent experiments performed in triplicate ±SE.

2.2. Western blot and immunofluorescence

Fractionation of Huh7 cells, analysis of fractions or total cellular proteins by SDS–PAGE, immunoblotting to detect HIF-1α, ARNT, actin, MAPK and phospho-MAPK and immunofluorescent microscopy were carried out as previously described [12–14]. All experiments requiring image analysis were performed at least three times and representative results are shown. Densitometric analysis of the bands in blots was performed with the public domain software for image analysis 'ImageJ' [15].

2.3. Cell viability assay

Huh7 cells (0.5×10^3/well) were seeded into 96-well plates and incubated for 24 h before being treated with 10 μM of kaempferol or with DMSO as solvent control for another 48 h under normoxic or hypoxic conditions. In order to keep the effective kaempferol concentration in the medium relatively constant (between 5 and 10 μM), cells were washed once with PBS and fresh kaempferol- or DMSO-containing culture medium was added every 8 h. At the end of the incubation period, viability was colorimetrically determined with the "CellTiter 96 Aqueous One Solution Cell Proliferation Assay" kit (Promega, WI, USA) according to the manufacturer's instructions. Values were normalized by control experiments in the absence of cells in 96-well plates supplied with culture medium and kaempferol or DMSO alone. Cell viability was expressed as percentage of control from 2 independent experiments performed in triplicate.

2.4. Statistical analysis

For IC_{50} calculation, the values obtained from luciferase activity experiments ($n = 12$) or densitometric analysis ($n = 3$) were expressed as percent (± standard error) and IC_{50} values were calculated by an online provided tool based on the four parameter logistic model (4PL) (BioDataFit 1.02, Chang Bioscience, Inc., http://www.changbioscience.com/stat/ec50.html). Statistical differences between two groups of data were assessed using the unpaired *t*-test in the SigmaPlot v. 9.0 software (Systat, IL, USA); $P < 0.05$ was considered to be significant.

3. Results

Huh7 cells, containing a hypoxia-responsive reporter plasmid, were exposed to hypoxic conditions (1% O_2) for 4 h in the presence or absence of 1–100 μM kaempferol. The transcriptional activity of hypoxia induced HIF-1 was effectively inhibited by kaempferol in a dose-dependent manner ($IC_{50} = 5.16 \pm 0.66$ μM; Fig. 1A). A similar effect was observed in kaempferol treated Huh7 cells when HIF-1α was induced by exposing the cells to DMOG, a hypoxia-mimetic agent that stabilizes HIF-1α by inhibiting its hydroxylation; only this time the inhibitory effect of kaempferol on HIF-1 activity was more profound ($IC_{50} = 0.835 \pm 0.45$ μM; Fig. 1B).

Since HIF-1 activity relies on the regulation of its alpha subunit, the expression levels of HIF-1α were examined by western blot analysis in Huh7 cells exposed to hypoxia. Fig. 2A demonstrates that hypoxically-induced HIF-1α proteins levels remained essentially unaltered in cells that were treated with up to 50 μM kaempferol, a condition that almost abolished HIF-1 activity (Fig. 1A), suggesting that kaempferol impairs the activation but not the expression of HIF-1α. Formation of active HIF-1 requires the presence of HIF-1α inside the nucleus. We, therefore, examined the subcellular distribution of HIF-1α by fractionating Huh7 cells that were previously subjected to hypoxia. As shown in Fig. 2B, increasing concentrations of kaempferol caused the reduction of HIF-1α levels in the nuclear fraction, while its cytoplasmic levels were respectively increased; suggesting that exposure of Huh7 cells to kaempferol inactivates HIF-1 by mislocalizing hypoxically-stabilized HIF-1α into the cytoplasm.

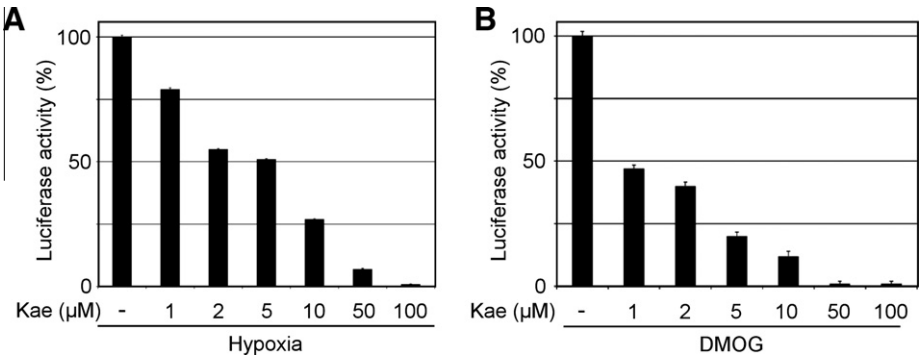

Fig. 1. Kaempferol inhibits HIF-1α activity in hypoxic Huh7 cells. (A) HIF-1 transcriptional activity in Huh7 cells incubated under hypoxic conditions (1% O_2) for 4 h in the presence of various concentrations of kaempferol (Kae). Data represent the mean (± standard error) of three independent experiments performed in triplicate and are expressed as % activity in relation to the value of untreated cells. (B) As in (A) but cells were exposed to 1 mM DMOG instead of hypoxia.

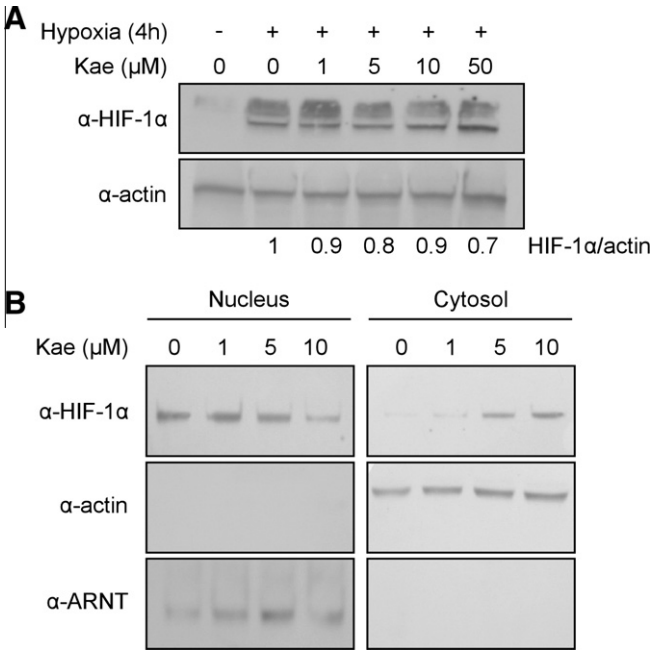

Fig. 2. Kaempferol treatment does not influence HIF-1α protein levels but affects its subcellular distribution. (A) Western blot analysis of total cell extracts from Huh7 cells treated for 4 h with various kaempferol concentrations (Kae) under hypoxia (1% O_2). The numbers underneath the panels represent the HIF-1α/actin protein levels ratio according to densitometric analysis of blots from three independent experiments. (B) Western blot analysis of nuclear (left panels) and cytoplasmic (right panels) fractions of Huh7 cells incubated as in (A). Actin and ARNT are used as loading and fractionation controls for the cytoplasmic and nuclear fractions, respectively.

To verify this, we performed immunofluorescence microscopy analysis of hypoxia-treated Huh7 cells. As shown in Fig. 3A, HIF-1α was exclusively nuclear in the absence of kaempferol but in the presence of increasing concentrations of kaempferol HIF-1α was gradually redistributed to the cytoplasm. Interestingly, at 5 μM kaempferol, a concentration corresponding to its IC_{50} for inhibition of HIF-1 activity, both western blotting and microscopy results show translocation of significant amounts of HIF-1α into the cytoplasm. Overall, our data suggest that kaempferol affects predominantly the regulation of HIF-1α nucleocytoplasmic transport.

We have previously reported that p44/42 MAPK plays an important role in HIF-1 regulation by controlling HIF-1α subcellular distribution. More specifically, phosphorylation by MAPK promotes nuclear accumulation of HIF-1α by blocking its CRM1-dependent nuclear export [12,13]. It is, therefore, possible that HIF-1α mislocalization caused by kaempferol in Huh7 cells may be due to inhibition of the MAPK pathway, which is active in these cells [2]. To test this hypothesis, we analysed p44/42 MAPK activation levels by determining its phosphorylation status in Huh7 cells in response to treatment with increasing kaempferol concentrations. As shown in Fig. 3B, immunoblotting reveals that kaempferol potently inhibits MAPK phosphorylation under hypoxic conditions in a dose-dependent manner. Importantly, the IC_{50} value for this inhibition (4.75 ± 0.26 μM) is almost identical to the IC_{50} for the inhibition of HIF-1 activity (5.16 μM), strongly indicating that the effect of kaempferol on HIF-1 activity and HIF-1α localization is MAPK-dependent.

As already described, HIF-1 activity is very important for the survival and proliferation of cells under hypoxia, a condition that often prevails in solid tumors. We could, therefore, expect that kaempferol may affect Huh7 viability under hypoxic conditions. To address this possibility, we measured survival of Huh7 cells after prolonged incubation (24–48 h) at 1% and 0.1% oxygen and in the presence of 10 μM kaempferol. To ensure constant exposure of the cells to effective concentrations of kaempferol, the medium was replaced every 8 h, time period that corresponds to the experimentally determined half-life of kaempferol in the culture (data not shown). In the absence of kaempferol, Huh7 cells exhibited similar proliferation under both normoxic and hypoxic conditions at 24 or 48 h (Fig. 4), although cells grew slightly better when deprived of oxygen. Culturing of Huh7 cells in the presence of 10 μM kaempferol did not result to significant inhibition of cell viability

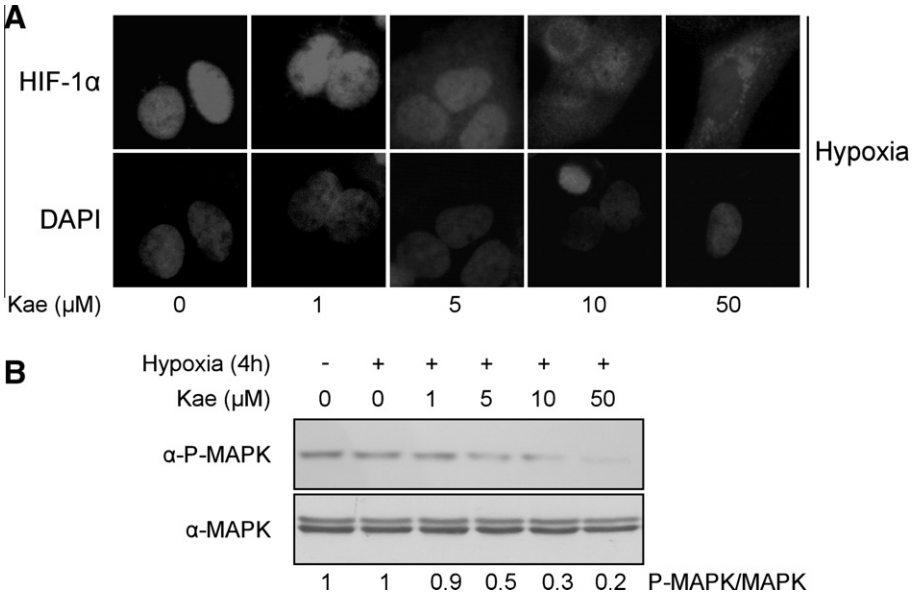

Fig. 3. Kaempferol impairs HIF-1α nuclear accumulation and p44/42 MAPK activation. (A) Huh7 cells were incubated under hypoxic conditions (1% O_2) in the presence of the indicated concentrations of kaempferol (Kae) for 4 h before processing for immunofluoresence microscopy with an anti-HIF-1α antibody. Nuclei are stained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI). (B) Western blot analysis of total cell extracts from Huh7 cells treated as in (A) using antibodies against the phosphorylated (top) or all (bottom) forms of p44/42 MAPK. The numbers below the panels represent the phospho-MAPK/MAPK levels ratio according to densitometric analysis of blots from three independent experiments.

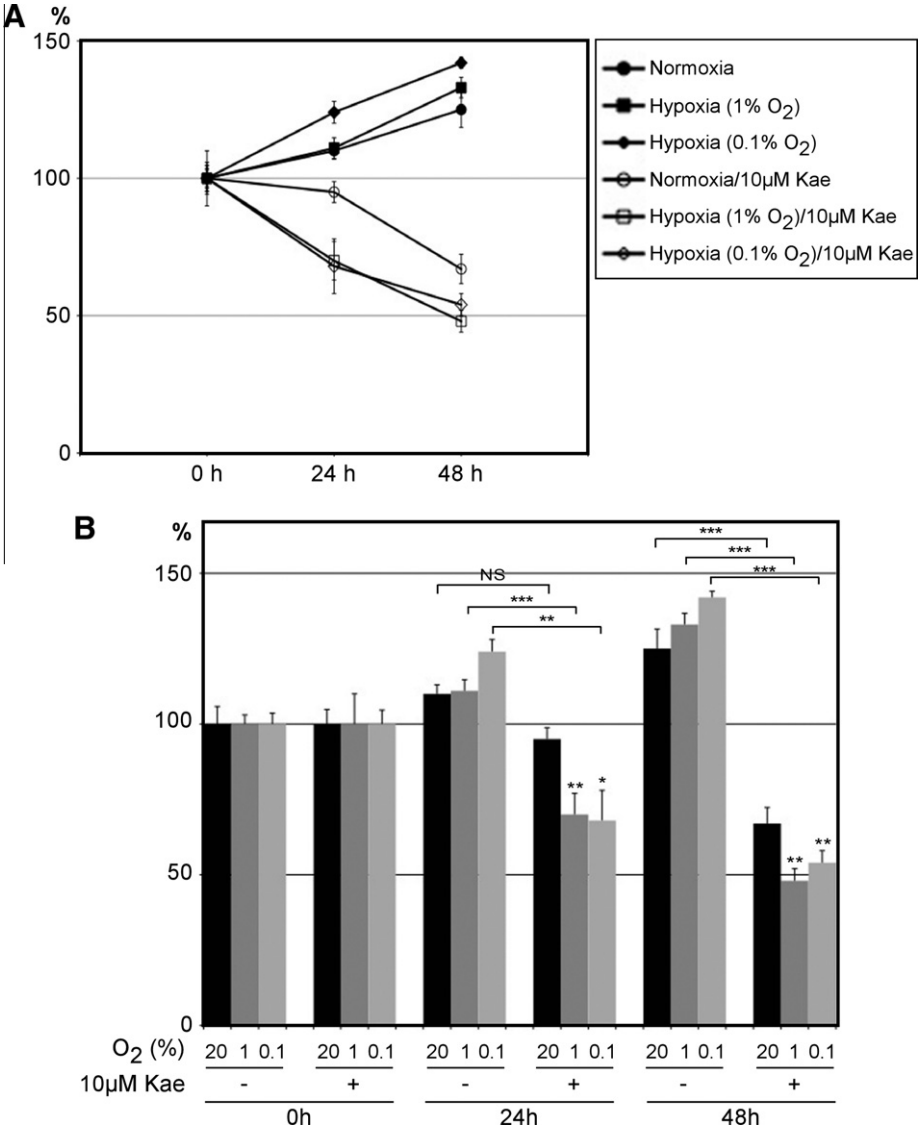

Fig. 4. Kaempferol inhibits viability of Huh7 cells under hypoxia. (A) Digitized graph of Huh7 cell survival under normoxic (circles) or hypoxic conditions (1% O_2 squares; 0.1% O_2 diamonds) and in the presence (empty) or absence (filled) of 10 μM of kaempferol (Kae). (B) Statistical significance of the data presented in (A). Data represent the mean (± standard error) of two independent experiments performed in triplicate and are expressed as percent of the initial value of cell number at time zero (NS: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$).

after 24 h in normoxia. In sharp contrast, treatment of the cells with kaempferol under hypoxia resulted in significant (approx. 40%) inhibition of their viability in both hypoxic conditions. At 48 h, inhibition of cell viability by kaempferol was observed at both normoxia and hypoxia. However, inhibition at hypoxia (approx. 65%) was still significantly higher than at normoxia (45%). These results reveal that Huh7 cells are considerably more sensitive to kaempferol treatment when exposed to hypoxic conditions, under which HIF-1 activity becomes important for their survival.

4. Discussion

This report shows, for the first time, that kaempferol is a potent inhibitor of hepatoma cancer cell viability under hypoxic conditions. It also provides evidence that this effect involves MAPK pathway inhibition and inactivation of HIF-1α by cytoplasmic mislocalization. Kaempferol may play a dual role in impairing cancer cell growth. Its mild effect on Huh7 cell survival under normal oxygen concentration is likely due to inhibition of the MAPK pathway, which is critical for cell proliferation. Under hypoxic conditions, however, when cell viability depends on HIF-1, kaempferol can exert a much stronger negative effect. This provides proof-of-principle for its potential use as an anti-HCC agent, since kaempferol will selectively target cancer cells, which are normally exposed to hypoxia. The potential of kaempferol to inhibit both MAPK and HIF-1 at low μM concentration (IC_{50} close to 5 μM) is far superior to the commonly used commercially available MAPK-pathway synthetic inhibitor PD98059, which is effective at a concentration of 50 μM or above [12–14].

Recent studies have shown the importance of HIF-1 inactivation as a means to treat human cancer since HIF-1 is a key factor of metabolic adaptation and reprogramming of cancer cells in the hypoxic microenvironment that is observed in many human tumors [8,16]. Suppression of HIF-1 may be even more beneficial when combined with other therapeutic strategies such as anti-angiogenic treatment. Successful suppression of angiogenesis can induce a phenotypic change in cancer cells that renders them more invasive and metastatic due to the establishment of severe hypoxia

[17]. This change could be avoided if HIF-1 induction under these conditions is simultaneously inhibited. It has indeed been shown that inhibition of HIF-1α expression by antisense cDNA stimulates the efficacy of doxorubicin on HCC cells by down-regulating VEGF transcription and enhancing apoptosis [18]. In addition, compounds that are natural derivatives, like silibinin, have exhibited anti-cancer properties by inhibiting HIF-1α synthesis in hepatoma cells [19].

Kaempferol was previously reported to affect cell survival in leukemic [20] and glioma cells [21], angiogenesis and tumor growth in ovarian cancer cells [22] and ovarian cancer incidence [23]. This report demonstrates the efficacy of kaempferol as a cytotoxic agent of hepatoma cancer cells in vitro at concentrations (5–10 μM) that are close to the plasma flavonoid concentration range achievable by dietary intake [23,24]. More importantly, the full potential of kaempferol to inhibit the viability of HCC cells occurs under oxygen concentrations (0.1–1% oxygen) that are physiologically more relevant to those inside a tumor growing in vivo. This is, to our knowledge, the first report demonstrating the biological effect of a flavonoid under low oxygen conditions, which minimize the possibility that the observed effects are attributed to artificial oxidative changes. Polyphenols, such as kaempferol, applied to cells growing under 20% oxygen, which is in reality a state of hyperoxia, can indeed exhibit pro-oxidant effects that greatly affect cell properties in a non-physiological way [25].

5. Conclusion

In conclusion, kaempferol can act as an HIF-1 inhibitor and antiproliferative agent under hypoxic conditions that prevail in solid tumors. As a naturally occurring dietary substance without known side effects, kaempferol could be a good candidate for further evaluation, as chemopreventive or therapeutic compound, in controlled prospective studies of HCC patients along or in combination with other established conservative and interventional therapies. These studies can then offer definite conclusions regarding the clinical efficiency of kaempferol or its derivatives as an additional weapon in the management of HCC.

Acknowledgments

We would like to thank all the members of the Laboratory of Biochemistry and Prof. G. Dalekos (Dept. of Medicine), in the Medical School. University of Thessaly, for their useful comments and suggestions.

References

[1] J. Bruix, J.M. Llovet, Major achievements in hepatocellular carcinoma, Lancet 373 (2009) 614–616.
[2] D.F. Calvisi, S. Ladu, A. Gorden, M. Farina, E.A. Conner, J.S. Lee, V.M. Factor, S.S. Thorgeirsson, Ubiquitous activation of Ras and Jak/Stat pathways in human HCC, Gastroenterology 130 (2006) 1117–1128.
[3] J.M. Llovet, J. Bruix, Molecular targeted therapies in hepatocellular carcinoma, Hepatology 48 (2008) 1312–1327.
[4] S.M. Wilhelm, C. Carter, L. Tang, D. Wilkie, A. McNabola, H. Rong, C. Chen, X. Zhang, P. Vincent, M. McHugh, Y. Cao, J. Shujath, S. Gawlak, D. Eveleigh, B. Rowley, L. Liu, L. Adnane, M. Lynch, D. Auclair, I. Taylor, R. Gedrich, A. Voznesensky, B. Riedl, L.E. Post, G. Bollag, P.A. Trail, BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis, Cancer Res. 64 (2004) 7099–7109.
[5] J.M. Llovet, S. Ricci, V. Mazzaferro, P. Hilgard, E. Gane, J.F. Blanc, A.C. de Oliveira, A. Santoro, J.L. Raoul, A. Forner, M. Schwartz, C. Porta, S. Zeuzem, L. Bolondi, T.F. Greten, P.R. Galle, J.F. Seitz, I. Borbath, D. Haussinger, T. Giannaris, M. Shan, M. Moscovici, D. Voliotis, J. Bruix, Sorafenib in advanced hepatocellular carcinoma, N. Engl. J. Med. 359 (2008) 378–390.
[6] X.Z. Wu, G.R. Xie, D. Chen, Hypoxia and hepatocellular carcinoma: The therapeutic target for hepatocellular carcinoma, J. Gastroenterol. Hepatol. 22 (2007) 1178–1182.
[7] G.L. Semenza, Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics, Oncogene 29 (2009) 625–634.
[8] E. Poon, A.L. Harris, M. Ashcroft, Targeting the hypoxia-inducible factor (HIF) pathway in cancer, Expert Rev. Mol. Med. 11 (2009) e26.
[9] G.W. Huang, L.Y. Yang, W.Q. Lu, Expression of hypoxia-inducible factor 1alpha and vascular endothelial growth factor in hepatocellular carcinoma: Impact on neovascularization and survival, World J. Gastroenterol. 11 (2005) 1705–1708.
[10] H. Tanaka, M. Yamamoto, N. Hashimoto, M. Miyakoshi, S. Tamakawa, M. Yoshie, Y. Tokusashi, K. Yokoyama, Y. Yaginuma, K. Ogawa, Hypoxia-independent overexpression of hypoxia-inducible factor 1alpha as an early change in mouse hepatocarcinogenesis, Cancer Res. 66 (2006) 11263–11270.
[11] D.F. Yao, H. Jiang, M. Yao, Y.M. Li, W.J. Gu, Y.C. Shen, L.W. Qiu, W. Wu, X.H. Wu, W.L. Sai, Quantitative analysis of hepatic hypoxia-inducible factor-1alpha and its abnormal gene expression during the formation of hepatocellular carcinoma, Hepatobiliary Pancreat. Dis. Int. 8 (2009) 407–413.
[12] I. Mylonis, G. Chachami, M. Samiotaki, G. Panayotou, E. Paraskeva, A. Kalousi, E. Georgatsou, S. Bonanou, G. Simos, Identification of MAPK phosphorylation sites and their role in the localization and activity of hypoxia-inducible factor-1alpha, J. Biol. Chem. 281 (2006) 33095–33106.
[13] I. Mylonis, G. Chachami, E. Paraskeva, G. Simos, A typical CRM1-dependent nuclear export signal mediates regulation of hypoxia-inducible factor-1alpha by MAPK, J. Biol. Chem. 283 (2008) 27620–27627.
[14] A. Triantafyllou, I. Mylonis, G. Simos, S. Bonanou, A. Tsakalof, Flavonoids induce HIF-1alpha but impair its nuclear accumulation and activity, Free Radic. Biol. Med. 44 (2008) 657–670.
[15] M.D. Abramoff, P.J. Magelhaes, S.J. Ram, Image Processing with Image, J. Biophot. Int. 11 (2004) 36–42.
[16] G. Kroemer, J. Pouyssegur, Tumor cell metabolism: cancer's Achilles' heel, Cancer Cell 13 (2008) 472–482.
[17] M.F. McCarty, J. Barroso-Aranda, F. Contreras, Practical strategies for suppressing hypoxia-inducible factor activity in cancer therapy, Med. Hypotheses 74 (2010) 789–797.
[18] F. Liu, P. Wang, X. Jiang, G. Tan, H. Qiao, H. Jiang, G.W. Krissansen, X. Sun, Antisense hypoxia-inducible factor 1alpha gene therapy enhances the therapeutic efficacy of doxorubicin to combat hepatocellular carcinoma, Cancer Sci. 99 (2008) 2055–2061.
[19] P. Garcia-Maceira, J. Mateo, Silibinin inhibits hypoxia-inducible factor-1alpha and mTOR/p70S6K/4E-BP1 signalling pathway in human cervical and hepatoma cancer cells: implications for anticancer therapy, Oncogene 28 (2009) 313–324.
[20] G. Marfe, M. Tafani, M. Indelicato, P. Sinibaldi-Salimei, V. Reali, B. Pucci, M. Fini, M.A. Russo, Kaempferol induces apoptosis in two different cell lines via Akt inactivation Bax and SIRT3 activation and mitochondrial dysfunction, J. Cell. Biochem. 106 (2009) 643–650.
[21] J.C. Jeong, M.S. Kim, T.H. Kim, Y.K. Kim, Kaempferol induces cell death through ERK and Akt-dependent down-regulation of XIAP and survivin in human glioma cells, Neurochem. Res. 34 (2009) 991–1001.
[22] H. Luo, G.O. Rankin, L. Liu, M.K. Daddysman, B.H. Jiang, Y.C. Chen, Kaempferol inhibits angiogenesis and VEGF expression through both HIF dependent and independent pathways in human ovarian cancer cells, Nutr. Cancer 61 (2009) 554–563.
[23] M.A. Gates, S.S. Tworoger, J.L. Hecht, I. De Vivo, B. Rosner, S.E. Hankinson, A prospective study of dietary flavonoid intake and incidence of epithelial ovarian cancer, Int. J. Cancer 121 (2007) 2225–2232.
[24] C. Manach, G. Williamson, C. Morand, A. Scalbert, C. Remesy, Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies, Am. J. Clin. Nutr 81 (2005) 230S–242S.
[25] B. Halliwell, Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies?, Arch Biochem. Biophys. 476 (2008) 107–112.