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The Flavonols Quercetin, Kaempferol, and Myricetin Inhibit Hepatocyte Growth Factor-Induced Medulloblastoma Cell Migration[1–3]

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Abstract

Medulloblastoma, the most common malignant brain tumor in children, is a highly metastatic disease, with up to 30% of children having evidence of disseminated disease at presentation. Recently, the hepatocyte growth factor (HGF) and its receptor, the tyrosine kinase Met, have emerged as key components of human medulloblastoma growth and metastasis, suggesting that inhibition of this pathway may represent an attractive target for the prevention and treatment of this disease. Using immunoblotting procedures, we observed that the dietary-derived flavonols quercetin, kaempferol, and myricetin inhibited HGF/Met signaling in a medulloblastoma cell line (DAOY), preventing the formation of actin-rich membrane ruffles and resulting in the inhibition of Met-induced cell migration in Boyden chambers. Furthermore, quercetin and kaempferol also strongly diminished HGF-mediated Akt activation. Interestingly, the inhibitory effects of quercetin on the tyrosine kinase receptor Met [half-maximal inhibitory effect (IC_{50}) of 12 μmol/L] or on the Met-induced activation of Akt (IC_{50} of 2.5 μmol/L) occurred at concentrations achievable through dietary approaches. These results highlight quercetin, kaempferol, and myricetin as dietary-derived inhibitors of Met activity and suggest that this inhibitory effect may contribute to the chemopreventive properties of these molecules. J. Nutr. 139: 646–652, 2009.

Introduction

Brain tumors are the second most common pediatric cancer, accounting for >26% of childhood cancer deaths (1). Medulloblastoma is the most common of these malignant tumors (2) and represents up to 40% of all cerebellar tumors in childhood (3). The treatment outcome for medulloblastoma is relatively low, resulting in a 5-y survival rate of 50–60% (4). Medulloblastoma is a highly metastatic disease (up to 30% of children have evidence of disseminated disease at presentation) (3) and despite whole brain and spinal radiation for prevention and dissemination, almost one-half of the patients die of early tumor recurrence (4). Furthermore, most survivors suffer from sequelae such as devastating cognitive effects, growth impairment as a result of hormone deficiency, early puberty development, and compromised spinal growth (5).

The targeting of tumor cell signaling pathways has recently been proposed as an alternative to conventional cytotoxic approaches for the treatment of medulloblastoma (5). Among these, tyrosine kinase receptors (RTK)[4] such as epidermal growth factor receptor (ERBB)-2, platelet-derived growth factor receptor, and the insulin-like growth factor receptor I are all associated with poor prognosis in medulloblastoma (4). ERBB-2 was particularly studied, because its expression was correlated with a more advanced metastatic stage (6) and phase I and II trials with erlotinib (an inhibitor of ERBB-2 signaling in human medulloblastoma cells both in vitro and in vivo) is actually underway in the Children's Oncology Group and the US Pediatric Brain Tumor Consortium (5). More recently, the hepatocyte growth factor (HGF) and its receptor, Met, have emerged as a new pathway involved in the growth of human medulloblastoma (7,8). Met activation through HGF binding is already known to play a role in the regulation of cell growth, morphogenesis, angiogenesis, and cell motility in a wide variety of human carcinomas and its expression correlates with poor prognosis (9). The importance of Met in tumor growth has led to the development of tyrosine

[4] Abbreviations used: EGCG, (-)-epigallocatechin gallate, ERBB, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; HGF, hepatocyte growth factor; IC_{50}, half-maximal inhibitory effect; PI3K, phosphatidylinositol-3-kinase; RTK, tyrosine kinase receptor.

kinase inhibitors and monoclonal antibodies that block the activation of Met and some of these compounds are already being tested in clinical trials (10).

In addition to these promising anticancer therapeutic approaches, considerable emphasis has lately been placed on chemoprevention, especially with naturally occurring products found in the diet. Abundant intake of a variety of fruit and nonstarchy vegetables has been proposed as a key lifestyle factor for the prevention of a wide variety of cancer types (11). Although the chemopreventive effects of fruits and vegetables remain incompletely understood, the National Cancer Institute has determined in laboratory studies that >1000 different phytochemicals possess cancer-preventive activity and it is estimated that there could be as much as 100 different of these phytochemicals per serving of vegetables (12). Among these, flavonoids are now recognized as potent inhibitors of an array of RTK such as ERBB-1 (13), ERBB-2 (14), platelet-derived growth factor receptor (15), insulin-like growth factor receptor-I (16), or the vascular endothelial growth factor receptor-2 (17) both in vitro or in vivo. Following ingestion, these molecules are bioavailable to a variety of tissues, including brain (18), and may thus have important chemopreventive properties (12). The most abundant flavonoids in the diet, flavonols, exhibit numerous biological and pharmacological effects, including anticancer-related properties (19). The main flavonols are kaempferol, myricetin, and quercetin (**Supplemental Fig. 1**), the latter being the most abundant bioflavonoid found in a variety of plant-based foods such as onions, apples, tea, broccoli, and red wine (20). Quercetin may also be found in the glycoside form, e.g. as quercitrin (Supplemental Fig. 1). By contrast, the distribution of kaempferol and myricetin is more restricted, with kaempferol being found primarily in caper, kale cress, and broccoli whereas myricetin is mainly present in fennel, parsley, and cranberries. Although some studies have suggested an important inhibitory potential of some flavonoids on the HGF/Met signaling pathway (21–23), the influence of flavonols on the Met receptor remains largely unknown.

Materials and Methods

Materials. Cell culture media were obtained from Wisent and serum was purchased from Hyclone Laboratories. Flavonols kaempferol and myricetin were obtained from Extrasynthèse, quercetin dihydrate and quercitrin were purchased from Sigma, and the flavanol (-)-epigallocatechin gallate (EGCG) was obtained from MP Biomedicals. Electrophoresis reagents were obtained from Bio-Rad. The anti-Met and anti-phospho-Met (Tyr 1234/1235) monoclonal antibodies and anti-Akt, anti-phospho-Akt (Ser 473), anti-extracellular signal-regulated kinase (ERK)-1/2, and anti-phospho-p44/42 MAPK (Thr 202/Tyr204) polyclonal antibodies were from Cell Signaling Technology. Anti-mouse and anti-rabbit horseradish peroxidase-linked secondary antibodies were purchased from Jackson ImmunoResearch Laboratories and enhanced chemiluminescence reagents were from PerkinElmer Life Sciences. Human recombinant HGF was obtained from R & D Systems, the Texas Red phalloidin from Sigma, and the Met kinase inhibitor SU11274 was purchased from EMD Chemicals.

Cell culture. The human DAOY medulloblastoma cell line was purchased from ATCC and was maintained in minimum essential medium containing 10% (v:v) bovine calf serum, 2 mmol/L glutamine, 100 kU/L penicillin, and 100 g/L streptomycin. Cells were incubated at 37°C with 95% air and 5% CO_2. For experimental purposes, DAOY were grown to confluence before overnight serum starvation without supplements. Cells were treated with vehicle or with the different compounds (flavonols and SU11274 diluted in dimethylsulfoxide or EGCG diluted in 100% ethanol) for 2 h and then stimulated with HGF.

Immunoblotting procedures. After treatment with HGF (50 μg/L, 3 min), DAOY were washed once with ice-cold PBS (pH 7.4) containing 1 mmol/L Na_3VO_4 and were incubated in this medium free of flavonoids and HGF for 1 h at 4°C. Equal amounts of protein (10 μg) from cell lysates were mixed in Laemmli sample buffer as described previously (15) and resolved by SDS-PAGE by either a 7.5% gel (Met detections) or 10% (Akt and ERK detections). The proteins were transferred onto polyvinylidene difluoride membranes, blocked overnight at 4°C in Tris-buffered saline/Tween 20 (147 mmol/L NaCl, 20 mmol/L Tris/HCl, pH 7.5, and 0.1% Tween 20) containing 5% (wt:v) milk powder, and probed with primary antibodies (Met, pMet, Akt, pAkt, ERK, pERK) for 1 h at room temperature. Immunoreactive bands were revealed after a 1-h incubation with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG, and the signals were visualized by enhanced chemiluminescence.

Migration assays. Transwells (8-μm pore size; Costar) were precoated with 0.15% gelatin in PBS by adding 600/100 μL in the lower/upper chambers overnight at 4°C. The Transwells were then washed with PBS and assembled into 24-well plates. The upper chamber of each Transwell was filled with 50 μL of cells (1.0×10^9 cells/L) and DAOY were allowed to adhere for 45 min. The monolayers were then treated for 2 h by adding 50 μL of 2-fold concentrated flavonoids (kaempferol, quercetin, myricetin, quercitrin, or EGCG) solution prepared in serum-free medium into the upper chamber and 600 μL of the compound solution (1×) into the lower chamber. After 2 h, HGF (20 μg/L) was added to the lower chamber as a chemoattractant. The plate was placed at 37°C in 5% CO_2/95% air for another 3 h. Cells that had migrated to the lower surface of the filters were fixed, stained, and migrations were then quantified as described previously (24).

Fluorescence and confocal microscopy. DAOY were plated on cover glasses coated with 0.15% gelatin-PBS; serum-starved overnight; treated with the vehicle, the flavonols (10 μmol/L), or SU11274 (1 μmol/L) for 2 h; and then stimulated (or not) with 50 μg/L HGF for 1 h. Cells were fixed with 3.7% formaldehyde for 15 min, permeabilized with 0.2% Triton X-100 for 5 min, and stained for the actin cytoskeleton with a 1/2500 dilution of Texas Red phalloidin (Sigma) for 30 min. Slides were mounted with Immuno-Fluore Mounting medium (MP Biomedicals) and staining was visualized and photographed using a Zeiss LSM 510 Meta confocal microscope.

Cell adhesion assays. Overnight, 96-well plates were precoated with 0.15% gelatin-PBS at 4°C. Wells were then blocked by adding a solution of PBS/bovine serum albumin 0.5% for 1 h at room temperature and then washed with 100 μL of adhesion buffer Eagle's minimum essential medium serum-free medium (0.2% bovine serum albumin, 15 mmol/L HEPES, and 0.12% $NaHCO_3$). Meanwhile, DAOY were harvested by incubation with 0.53 mmol/L EDTA in PBS, pH 7.2, for 10–15 min at 37°C. EDTA was neutralized by the addition of adhesion buffer and cells were centrifuged at $1000 \times g$; 3 min. Then 5×10^4 cells were added per well and allowed to adhere on gelatin or plastic with or without the vehicle, the flavonols, or SU11274 for 2 h at 37°C. Cells were then fixed, washed, stained, and lysed (25). Spectrophotometric absorbance was then measured at 600 nm.

Statistical analysis. The data are presented as means ± SEM and statistical analysis was performed using 1-way ANOVA with a post hoc Dunnett's test. Differences with $P < 0.05$ were considered significant.

Results

Quercetin, myricetin, and kaempferol inhibit Met phosphorylation induced by HGF in DAOY. We first examined the effect of dietary flavonols on the HGF-induced tyrosine phosphorylation of Met using a monoclonal antibody that recognizes the phosphorylation of 2 tyrosine residues (1234/1235) that are required for complete activation of the HGF/Met signaling pathway (26). Quiescent DAOY were incubated for 16 h in

serum-free medium, treated with the vehicle or the indicated concentration of flavonols (quercitrin, quercetin, myricetin, and kaempferol) for 2 h, and then stimulated with human recombinant HGF (50 μg/L) for 3 min. The green tea catechin EGCG (Supplemental Fig. 1) was used as a positive control, because this polyphenol successfully inhibits HGF signaling in the immortalized, nontumorigenic breast (MCF10A), in the invasive breast carcinoma (MDA-MB-231) (21), and in the hypopharyngeal carcinoma (FaDu) cell lines (23). Quercetin, myricetin, and kaempferol dramatically reduced the phosphorylation of Met induced by HGF at a concentration of 20 μmol/L, whereas quercitrin had no effect (**Fig. 1**, top panels). Blotting the lysates with an antibody directed against Met showed that the molecules did not affect the amount of this receptor in the treated cells (Fig. 1, middle panels). The half-maximal inhibitory effect (IC_{50}) of the various flavanols on HGF-induced Met phosphorylation was 12 μmol/L for quercetin and ~6 μmol/L for myricetin and kaempferol. However, quercitrin did not inhibit HGF-induced Met phosphorylation even at 20 μmol/L (Fig. 1, top panels).

Low concentrations of quercetin and kaempferol inhibit Akt phosphorylation induced by HGF in DAOY. Medulloblastoma is a highly metastatic cancer that is disseminated in up to 43% of patients (3). Besides its role in cell survival and cell proliferation, Met signaling is also a potent activator of the cell invasive growth capacities (9). Met activation through HGF association results in Akt and ERK-1/2 phosphorylation, thereby promoting cell migration (10). To investigate whether the inhibition of Met tyrosine phosphorylation by flavonols also results in the inhibition of downstream intracellular signaling events, we examined the effect of these molecules on the HGF-induced Akt and ERK-1/2 phosphorylation. DAOY were serum-starved for 16 h before a 2-h treatment with the vehicle, the flavonols, or EGCG as a positive control. The treatment was followed by a 3-min stimulation with human recombinant HGF (50 μg/L). Quercetin and kaempferol, at very low concentrations, successfully inhibited Akt phosphorylation induced by the activation of Met signaling (**Fig. 2**). In fact, the inhibition of Akt phosphorylation occurs with an IC_{50} of 2.5 μmol/L for quercetin and 5 μmol/L for kaempferol, whereas quercitrin and myricetin did not inhibit this process. The inhibition of Akt phosphorylation by quercetin was lower than that observed with EGCG (IC_{50} of 5 μmol/L). In addition, although EGCG successfully inhibited ERK-1/2 phosphorylation, as previously reported for HGF-induced Met activation in 2 breast cell lines (21), the flavonols did not modulate ERK-1/2 phosphorylation induced by Met activation (**Supplemental Fig. 2**).

Flavonols myricetin, kaempferol, and quercetin inhibit HGF-mediated DAOY cell migration. The serine/threonine kinase Akt is an important mediator of cell migration and invasion in various cell types (27), including medulloblastoma (5). Because quercetin and kaempferol inhibited HGF-induced Met and Akt phosphorylation, we studied the effect of these molecules on DAOY migration. Cells were allowed to adhere to gelatin-coated Transwells and were incubated for 2 h with different concentrations of flavonols before the addition of HGF (20 μg/L) to the lower chambers. Under these conditions, myricetin, kaempferol, and quercetin inhibited HGF-induced migration of DAOY, but quercitrin had no effect (**Fig. 3**). We next compared the efficacy of myricetin, kaempferol, and quercetin in inhibiting DAOY migration to SU11274, a Met-specific small molecule inhibitor that competes with the binding
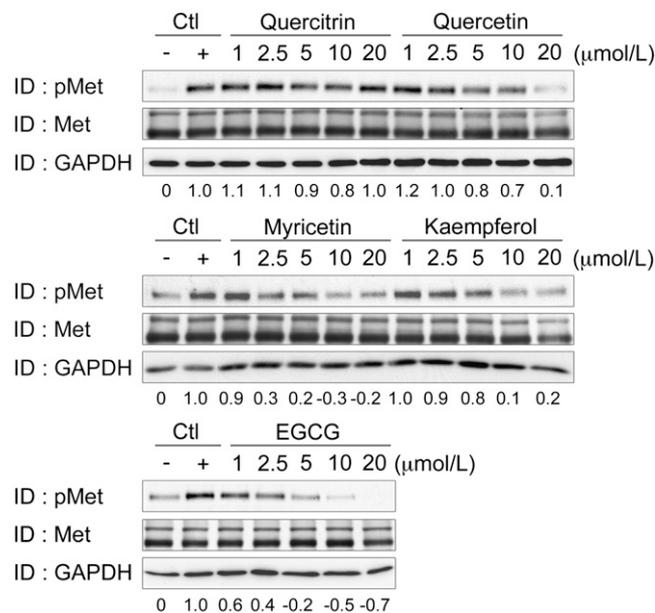

FIGURE 1 Quercetin, myricetin, and kaempferol inhibit Met phosphorylation induced by HGF in DAOY. Quiescent DAOY were incubated in serum-free medium for 16 h and then treated with the vehicle, quercitrin, quercetin, myricetin, kaempferol, or EGCG with the indicated concentrations of the molecules for 2 h. Cells were stimulated with recombinant HGF (50 μg/L) for 3 min. Cell lysates were prepared and analyzed by immunoblotting for phosphorylated Met and total Met. GAPDH protein levels served as an internal standard. Values indicate the ratio of phosphorylated Met:total Met protein.
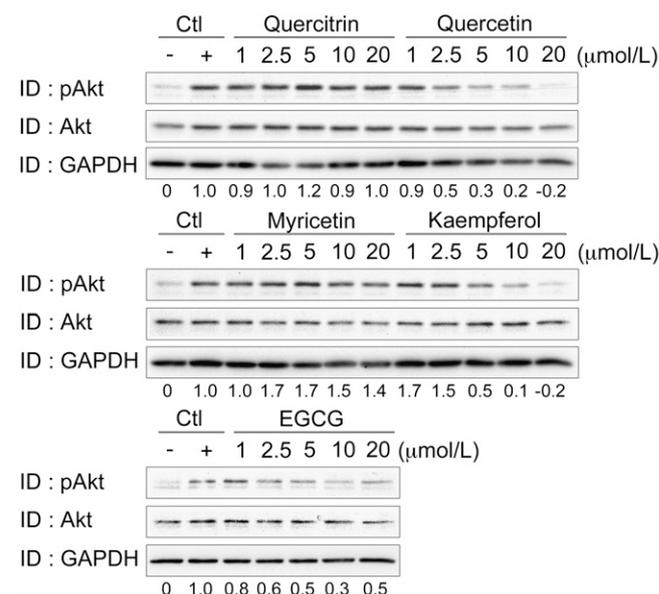

FIGURE 2 Low concentrations of quercetin and kaempferol inhibit Akt phosphorylation induced by HGF in DAOY, which were serum-starved for 16 h and then treated with the vehicle, quercitrin, quercetin, myricetin, kaempferol, or EGCG at the indicated concentrations for 2 h. Cells were stimulated with recombinant HGF (50 μg/L) for 3 min. Cell lysates were prepared and analyzed by immunoblotting for phosphorylated Akt and total Akt. Total Akt and GAPDH protein levels in cell lysates were detected as controls. Values indicate the ratio of phosphorylated Akt:total Akt protein.
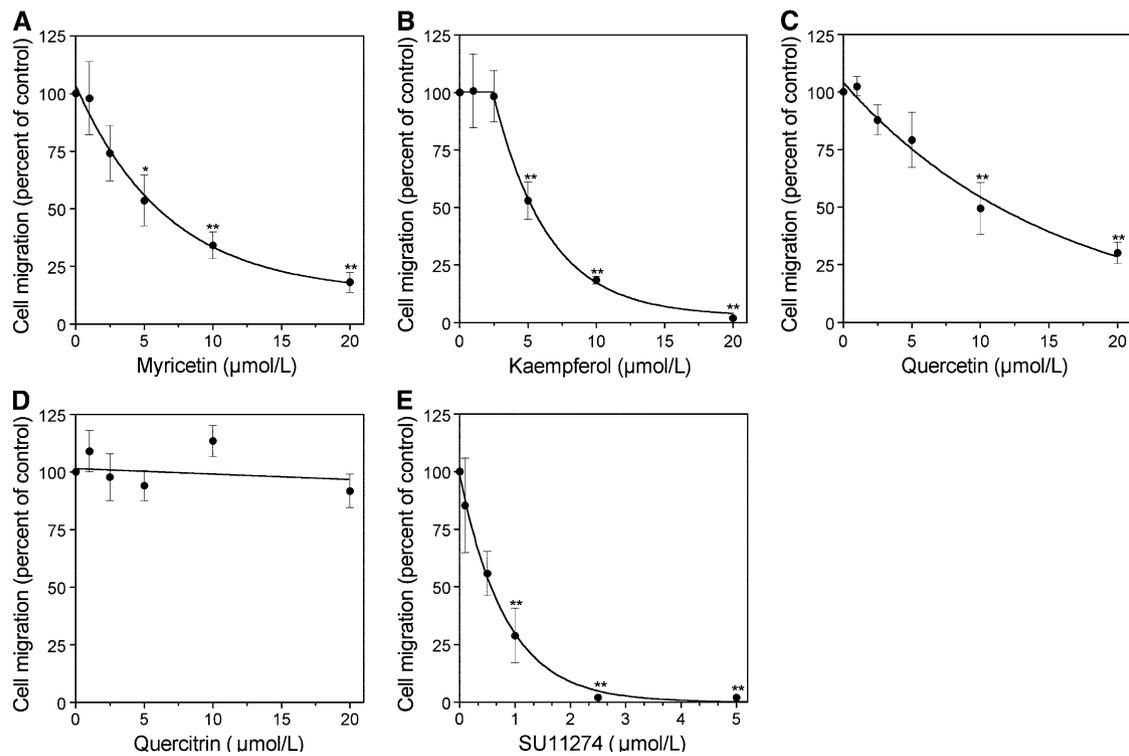
FIGURE 3 Flavonols myricetin, kaempferol, and quercetin inhibit HGF-mediated DAOY cell migration. DAOY cells were pretreated with the different flavonols, (*A*) myricetin, (*B*) kaempferol, (*C*) quercetin, and (*D*) quercitrin, at various concentrations for 2 h. Migration was initiated by adding 20 μg/L of recombinant HGF for 3 h. The same protocol was used for the specific Met kinase inhibitor (*E*) SU11274. Migration was quantified by counting the cells that crossed the membrane to the lower side of the filter with optical microscopy at magnification $\times$50. The number of cells that migrated was compared with that observed with untreated cells. Values are means of at least 3 independent experiments. Asterisks indicate different from control: * $P < 0.05$; ** $P < 0.01$.

of ATP to the kinase domain of the receptor (28). SU11274 was 10-fold more effective in the inhibition of HGF-mediated migration, with an IC_{50} of ~0.5 μmol/L compared with an IC_{50} of ~5 μmol/L for myricetin and kaempferol, and 20-fold more effective than quercetin (IC_{50} of 10 μmol/L) (Fig. 3). These results indicate that myricetin, kaempferol, and quercetin, naturally occurring molecules, are within one order of magnitude as efficient as SU11274 at inhibiting HGF-induced DAOY migration.

Treatment with the flavonols kaempferol, quercetin, and myricetin or with the Met kinase inhibitor SU11274 prevents morphological changes induced by HGF in DAOY. Upon HGF stimulation, Met activation induces several biological responses such as the activation of the cell invasive program, which results in cell morphological changes such as actin reorganization (29). We sought to investigate whether treatments of the cells with flavonols modified DAOY morphology using confocal microscopy. DAOY grown on gelatin-coated coverslips had a relatively uniform actin repartition as visualized by phalloidin staining (**Fig. 4**). Stimulation of DAOY with 50 μg/L HGF for 1 h caused the formation of actin-rich membrane ruffles at the cell periphery (indicated by arrows), a key event in cell migration, and the formation of these ruffles was inhibited by a 2-h treatment with SU11274 prior to HGF stimulation. Interestingly, preincubation of the cells with kaempferol, quercetin, or myricetin also inhibited actin relocalization induced by HGF, whereas quercitrin had no effect. Overall, these data indicate that the inhibition of the HGF signaling pathway by the flavonols kaempferol, quercetin, and myricetin prevent the
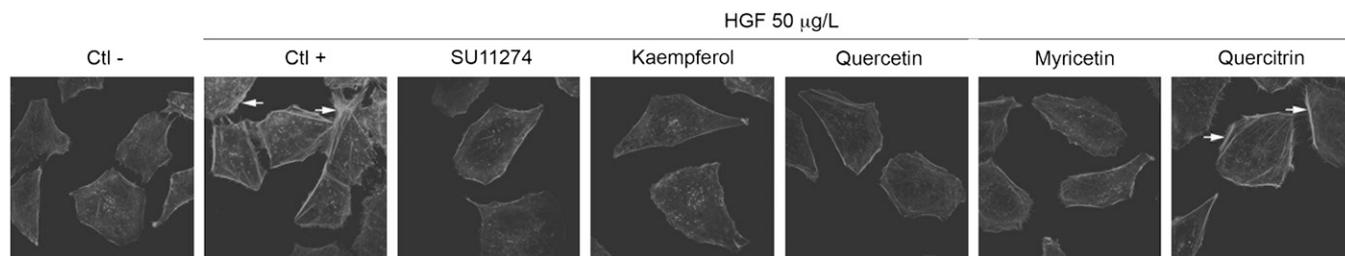

FIGURE 4 Kaempferol, quercetin, and myricetin prevent morphological changes induced by HGF in DAOY. DAOY (2 $\times$ 10^4 cells/well) were seeded onto gelatin-coated coverslips in a 24-well plate. Cells were serum-starved overnight, treated for 2 h with the vehicle or the different compounds, and then stimulated with 50 μg/L HGF for 1 h at 37°C. Cells were fixed and stained with Texas Red-conjugated phalloidin for actin. Representative cell images were obtained by confocal microscopy.

reorganization of the actin cytoskeleton and therefore interferes with the morphological changes induced by HGF, leading to the inhibition of medulloblastoma cell migration.

Discussion

In this study, we showed that low concentrations of quercetin, myricetin, and kaempferol, but not quercitrin (quercetin-3-O-α-rhamnoside), inhibit HGF-dependent phosphorylation of Met in DAOY. To the best of our knowledge, this is the first study to highlight the inhibitory effect of flavonols on this important RTK. Because the HGF-induced Met activation was reported to be inhibited in hepatoma cells with the flavone luteolin (22) and in breast epithelial cells with the green tea catechins EGCG and (-)-epicatechin gallate (21), these results strongly suggest that this RTK may represent a key target of dietary-derived chemopreventive polyphenols. The inhibitory effect of quercetin and kaempferol was associated with an impairment of Akt phosphorylation, a key downstream signaling event involved in the cellular effects of Met. However, myricetin did not inhibit Akt phosphorylation even though it inhibited HGF-induced Met activation, thereby suggesting that the inhibition of other intracellular kinases involved in Akt activation are likely to be involved in the effect of quercetin and kaempferol on this pathway.

Quercetin is known to act as a potent inhibitor of the phosphatidylinositol-3-kinase (PI3K), which is essential for Akt activation, and its structure was used as a model in the development of the PI3K selective inhibitor LY294002 (30). Its effect on Akt activation may thus be the sum of an effect on the upstream signal (Met and PI3K inhibition) and on the Akt phosphorylation (31). The loss of a hydroxyl group at position 3′ in the B ring of quercetin (giving kaempferol) did not alter the potential in inhibiting both Met and Akt activation, whereas the addition of a hydroxyl group at position 5′ in the B ring (giving myricetin) kept the Met-specific inhibition while losing the Akt inhibition (Supplemental Fig. 1). Furthermore, quercetin glycosylation (giving quercitrin) inhibits its activity against Met and Akt activation, suggesting that the sugar moiety interferes with quercetin potential. Although the exact mechanisms underlying the inhibitory effect of these flavonols on the HGF-induced Met and Akt activation in DAOY remains to be determined, previous work has demonstrated that quercetin binds directly to PI3K in the ATP-binding pocket (32) and the green tea catechin EGCG inhibits RTK activation by competing for the ATP-binding pocket (33). It is thus tempting to speculate that a similar mechanism is responsible for flavonol and EGCG activity against Met activation. Interestingly, no effect on the phosphorylation of the p42^{MAPK} and p44^{MAPK} forms (ERK-1/2) was observed in DAOY cells. These results stand in contrast to those obtained by other groups who showed a decrease of ERK phosphorylation in JB6 mouse epidermal cells (34) or an increase in ERK activity in A549 lung cancer cells (35) with quercetin. These discrepancies are likely due to differences in experimental conditions, notably the use of different cell types.

Importantly, the inhibition of Met by quercetin, myricetin, and kaempferol was correlated with an inhibition of DAOY migration, a crucial event implicated in medulloblastoma metastasis. Interestingly, the Met kinase inhibitor SU11274 was only between 10 and 20 times more efficient than flavonols in inhibiting migration, suggesting that low flavonol concentrations may have an impact on cell migration in vivo. Furthermore, all flavonols (except quercitrin) as well as SU11274 prevent morphological changes induced by HGF in the DAOY

cell migration process, suggesting that the inhibition of Met impairs cytoskeleton reorganization events underlying migration. However, flavonols do not antagonize the cell migration process through the upregulation of cell adhesion (data not shown) as previously demonstrated with EGCG in DAOY (25).

Dietary flavonol intake is estimated to be between 20 and 35 mg/d, of which quercetin represents the predominant flavonol (36). Quercetin naturally occurs as a glucoside or as various glycoside forms that are either hydrolyzed by intestinal enzymes or by the colonic microflora before they can be absorbed (37). Quercetin is of particular interest, because micromolar plasma concentration is achievable through diet. Graefe et al. (38) showed that an onion supplement consisting of 160 g stewed and homogenized onions, which provided 331 μmol/L of quercetin glucosides (equivalent to 100 mg quercetin), was sufficient to observe a quercetin plasma peak concentration of 7.6 μmol/L. Furthermore, one characteristic feature of quercetin bioavailability is that the elimination of quercetin metabolites is quite slow, with reported half-lives ranging from 11 to 28 h. This is particularly interesting, because this could favor accumulation in plasma with repeated intakes (36). These observations thus suggest that the inhibitory effect of quercetin on the RTK Met or on the Met-induced Akt activation occurs at concentrations achievable through dietary approaches (IC$_{50}$ of 10 μmol/L for Met and 2.5 μmol/L for Akt).

Importantly, in in vitro and in situ models of the blood-brain barrier, it was demonstrated that quercetin was able to enter different regions of the brain as a substrate of P-glycoprotein, a blood-brain barrier efflux transporter (39). This is in agreement with animal feeding studies that provide evidence that other flavonoids, such as the tea flavanol EGCG, may access the brain following oral administration to mice (40). Rats fed blackberry (41) or blueberry (42) extracts rich in anthocyanidins had intracerebral localization of the molecules. Together, these results suggest that quercetin, among other dietary flavonoids, may access the brain after oral ingestion in humans. Although the potential chemopreventive effects of polyphenols on pediatric brain tumor development remain poorly understood, it is noteworthy that a good maternal diet during pregnancy, including high consumption of fruits and vegetables, had a strong protective dose-response relation on the risk of pediatric brain tumors (43), whereas a high consumption of unhealthy foods, such as French fries, had a significant increased odds ratio (44). Our results also suggest that, given the high rate of cancer recurrence within 5 y for medulloblastoma survivors (~40%), a diet rich in flavonol-containing foods may play a crucial role in the secondary prevention of these tumors.

The use of drug combinations to circumvent tumor resistance is a well-established principle of cancer therapy (45). Recent advances indicate that Met and EGFR in glioma cells act as independent and redundant inputs to intracellular signaling networks, suggesting that single-target inhibition of either Met or EGFR would be ineffective (46). Because EGFR and now Met are implicated in medulloblastoma aggressiveness, phytochemicals such as quercetin that target both RTK (47) may thus circumvent this limitation and inhibit tumor cell progression more effectively. Furthermore, quercetin-rich foods also contain plenty of other phytochemicals that may act synergistically with quercetin and increase its chemopreventive activity. For example, broccoli, a rich source of quercetin, also contains high levels of sulforaphane, an isothiocyanate that inhibits angiogenesis through the inhibition of metalloproteinase-9-activated human brain microvascular endothelial cell migration and tubulogenesis (48) and induces medulloblastoma cell apoptosis (49).

In summary, these results suggest that flavonols, at concentrations achievable through diet, inhibit DAOY cell migration by affecting both Met and Akt (but not on ERK-1/2) as well as the cell actin cytoskeleton and that these effects may prevent invasion and metastasis by these cells. Further studies aimed at the identification of the beneficial effects of flavonols on medulloblastoma growth in vivo should provide interesting information on the clinical usefulness of these dietary-derived molecules in the prevention of pediatric brain tumors.

Literature Cited

1. Legler JM, Ries LAG, Smith MA, Warren JL, Heineman EF, Kaplan RS, Linet MS. Brain and other central nervous system cancers: recent trends in incidence and mortality. J Natl Cancer Inst. 1999;92:1382–90.

2. Polkinghorn WR, Tarbell NJ. Medulloblastoma: tumorigenesis, current clinical paradigm, and efforts to improve risk stratification. Nat Clin Pract Oncol. 2007;4:295–304.

3. Crawford JR, MacDonald TJ, Packer RJ. Medulloblastoma in childhood: new biological advances. Lancet Neurol. 2007;6:1073–85.

4. Rossi A, Caracciolo V, Russo G, Reiss K, Giordano A. Medulloblastoma: from molecular pathology to therapy. Clin Cancer Res. 2008;14:971–6.

5. Gilbertson RJ. Medulloblastoma: signalling a change in treatment. Lancet Oncol. 2004;5:209–18.

6. Gilbertson RJ, Clifford SC, MacMeekin W, Meekin W, Wright C, Perry RH, Kelly P, Pearson AD, Lunec J. Expression of the ErbB-neuregulin signaling network during human cerebellar development: implications for the biology of medulloblastoma. Cancer Res. 1998;58:3932–41.

7. Li Y, Guessous F, Johnson EB, Eberhart CG, Li X-N, Shu Q, Fan S, Lal B, Laterra J, et al. Functional and molecular interactions between the HGF//c-Met pathway and c-Myc in large-cell medulloblastoma. Lab Invest. 2007;88:98–111.

8. Li Y, Lal B, Kwon S, Fan X, Saldanha U, Reznik TE, Kuchner EB, Eberhart C, Laterra J, et al. The scatter factor/hepatocyte growth factor: c-Met pathway in human embryonal central nervous system tumor malignancy. Cancer Res. 2005;65:9355–62.

9. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. Nat Rev Mol Cell Biol. 2003;4:915–25.

10. Migliore C, Giordano S. Molecular cancer therapy: can our expectation be MET? Eur J Cancer. 2008;44:641–51.

11. World Cancer Research Fund/American Institute for Cancer Food Research. Food, nutrition, physical activity and the prevention of cancer: a global perspective. Washington, DC: World Cancer Research Fund/American Institute for Cancer Food Research; 2007.

12. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer. 2003;3:768–80.

13. Sah JF, Balasubramanian S, Eckert RL, Rorke EA. Epigallocatechin-3-gallate inhibits epidermal growth factor receptor signaling pathway: evidence for direct inhibition of erk1/2 and akt kinases. J Biol Chem. 2004;279:12755–62.

14. Pianetti S, Guo S, Kavanagh KT, Sonenshein GE. Green tea polyphenol epigallocatechin-3 gallate inhibits Her-2/Neu signaling, proliferation, and transformed phenotype of breast cancer cells. Cancer Res. 2002; 62:652–5.

15. Lamy S, Beaulieu E, Labbe D, Bedard V, Moghrabi A, Barrette S, Gingras D, Beliveau R. Delphinidin, a dietary anthocyanidin, inhibits platelet derived growth factor ligand/receptor (PDGF/PDGFR) signaling. Carcinogenesis. 2008;29:1033–41.

16. Adhami VM, Siddiqui IA, Ahmad N, Gupta S, Mukhtar H. Oral consumption of green tea polyphenols inhibits insulin-like growth factor-i-induced signaling in an autochthonous mouse model of prostate cancer. Cancer Res. 2004;64:8715–22.

17. Lamy S, Gingras D, Beliveau R. Green tea catechins inhibit vascular endothelial growth factor receptor phosphorylation. Cancer Res. 2002; 62:381–5.

18. Spencer JP. Flavonoids: modulators of brain function? Br J Nutr. 2008;99 Suppl 1:ES60–77.

19. Middleton E Jr, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev. 2000;52:673–751.

20. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. J Nutr. 2000;130:S2073–85.

21. Bigelow RLH, Cardelli JA. The green tea catechins, (-)-Epigallocatechin-3-gallate (EGCG) and (-)-Epicatechin-3-gallate (ECG), inhibit HGF// Met signaling in immortalized and tumorigenic breast epithelial cells. Oncogene. 2006;25:1922–30.

22. Lee W-J, Wu L-F, Chen W-K, Wang C-J, Tseng T-H. Inhibitory effect of luteolin on hepatocyte growth factor/scatter factor-induced HepG2 cell invasion involving both MAPK/ERKs and PI3K-Akt pathways. Chem Biol Interact. 2006;160:123–33.

23. Lim YC, Park HY, Hwang HS, Kang SU, Pyun JH, Lee MH, Choi EC, Kim CH. (-)-Epigallocatechin-3-gallate (EGCG) inhibits HGF-induced invasion and metastasis in hypopharyngeal carcinoma cells. Cancer Lett. 2008;271:140–52.

24. Lamy S, Lafleur R, Bedard V, Moghrabi A, Barrette S, Gingras D, Beliveau R. Anthocyanidins inhibit migration of glioblastoma cells: structure-activity relationship and involvement of the plasminolytic system. J Cell Biochem. 2007;100:100–11.

25. Pilorget A, Berthet V, Luis J, Moghrabi A, Annabi B, Beliveau R. Medulloblastoma cell invasion is inhibited by green tea (-)epigallocatechin-3-gallate. J Cell Biochem. 2003;90:745–55.

26. Longati P, Bardelli A, Ponzetto C, Naldini L, Comoglio PM. Tyrosines 1234–1235 are critical for activation of the tyrosine kinase encoded by the MET proto-oncogene (HGF receptor). Oncogene. 1994;9:49–57.

27. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007;129:1261–74.

28. Wang X, Le P, Liang C, Chan J, Kiewlich D, Miller T, Harris D, Sun L, Rice A, et al. Potent and selective inhibitors of the Met [hepatocyte growth factor/scatter factor (HGF/SF) receptor] tyrosine kinase block HGF/SF-induced tumor cell growth and invasion. Mol Cancer Ther. 2003;2:1085–92.

29. Soldati C, Biagioni S, Poiana G, Augusti-Tocco G. beta-Catenin and actin reorganization in HGF/SF response of ST14A cells. J Neurosci Res. 2008;86:1044–52.

30. Vlahos CJ, Matter WF, Hui KY, Brown RF. A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H−1-benzopyran-4-one (LY294002). J Biol Chem. 1994;269:5241–8.

31. Spencer JP, Rice-Evans C, Williams RJ. Modulation of pro-survival Akt/protein kinase B and ERK1/2 signaling cascades by quercetin and its in vivo metabolites underlie their action on neuronal viability. J Biol Chem. 2003;278:34783–93.

32. Walker EH, Pacold ME, Perisic O, Stephens L, Hawkins PT, Wymann MP, Williams RL. Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. Mol Cell. 2000;6:909–19.

33. Li M, He Z, Ermakova S, Zheng D, Tang F, Cho YY, Zhu F, Ma WY, Sham Y, et al. Direct inhibition of insulin-like growth factor-I receptor kinase activity by (-)-epigallocatechin-3-gallate regulates cell transformation. Cancer Epidemiol Biomarkers Prev. 2007;16:598–605.

34. Lee KW, Kang NJ, Heo YS, Rogozin EA, Pugliese A, Hwang MK, Bowden GT, Bode AM, Lee HJ, et al. Raf and MEK protein kinases are direct molecular targets for the chemopreventive effect of quercetin, a major flavonol in red wine. Cancer Res. 2008;68:946–55.

35. Nguyen TT, Tran E, Nguyen TH, Do PT, Huynh TH, Huynh H. The role of activated MEK-ERK pathway in quercetin-induced growth inhibition and apoptosis in A549 lung cancer cells. Carcinogenesis. 2004;25:647–59.

36. Manach C, Williamson G, Morand C, Scalbert A, Remesy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr. 2005;81:S230–42.

37. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004;79:727–47.

38. Graefe EU, Wittig J, Mueller S, Riethling AK, Uehleke B, Drewelow B, Pforte H, Jacobasch G, Derendorf H, et al. Pharmacokinetics and bioavailability of quercetin glycosides in humans. J Clin Pharmacol. 2001;41:492–9.

39. Youdim KA, Qaiser MZ, Begley DJ, Rice-Evans CA, Abbott NJ. Flavonoid permeability across an in situ model of the blood-brain barrier. Free Radic Biol Med. 2004;36:592–604.

40. Suganuma M, Okabe S, Oniyama M, Tada Y, Ito H, Fujiki H. Wide distribution of [3H](-)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. Carcinogenesis. 1998;19:1771–6.

41. Talavera S, Felgines C, Texier O, Besson C, Gil-Izquierdo A, Lamaison JL, Remesy C. Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. J Agric Food Chem. 2005;53:3902–8.

42. Andres-Lacueva C, Shukitt-Hale B, Galli RL, Jauregui O, Lamuela-Raventos RM, Joseph JA. Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. Nutr Neurosci. 2005;8: 111–20.

43. Bunin GR, Kuijten RR, Buckley JD, Rorke LB, Meadows AT. Relation between maternal diet and subsequent primitive neuroectodermal brain tumors in young children. N Engl J Med. 1993;329:536–41.

44. Bunin GR, Kushi LH, Gallagher PR, Rorke-Adams LB, McBride ML, Cnaan A. Maternal diet during pregnancy and its association with medulloblastoma in children: a children's oncology group study (United States). Cancer Causes Control. 2005;16:877–91.

45. Dancey JE, Chen HX. Strategies for optimizing combinations of molecularly targeted anticancer agents. Nat Rev Drug Discov. 2006;5:649–59.

46. Stommel JM, Kimmelman AC, Ying H, Nabioullin R, Ponugoti AH, Wiedemeyer R, Stegh AH, Bradner JE, Ligon KL, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. Science. 2007;318:287–90.

47. Fridrich D, Teller N, Esselen M, Pahlke G, Marko D. Comparison of delphinidin, quercetin and (-)-epigallocatechin-3-gallate as inhibitors of the EGFR and the ErbB2 receptor phosphorylation. Mol Nutr Food Res. 2008;52:815–22.

48. Annabi B, Rojas-Sutterlin S, Laroche M, Lachambre MP, Moumdjian R, Beliveau R. The diet-derived sulforaphane inhibits matrix metalloproteinase-9-activated human brain microvascular endothelial cell migration and tubulogenesis. Mol Nutr Food Res. 2008;52:692–700.

49. Gingras D, Gendron M, Boivin D, Moghrabi A, Theoret Y, Beliveau R. Induction of medulloblastoma cell apoptosis by sulforaphane, a dietary anticarcinogen from Brassica vegetables. Cancer Lett. 2004;203:35–43.