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Medulloblastoma Cell Invasion Is Inhibited by Green Tea (−)Epigallocatechin-3-Gallate

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Abstract Epigallocatechin-3-gallate (EGCG), the major green tea polyphenol, can reach the brain following oral intake and could thus act as an anti-tumoral agent targeting several key steps of brain cancer cells invasive activity. Because integrin-mediated extracellular matrix recognition is crucial during the cell adhesion processes involved in carcinogenesis, we have investigated the effects of EGCG on different cellular integrins of the pediatric brain tumor-derived medulloblastoma cell line DAOY. Using flow cytometry, we report the levels of expression of several cell surface integrins in DAOY. These include high expression of α2, α3, and β1 integrins, as well as αv and β3 integrins. Moreover, we provide evidence that EGCG can antagonize DAOY cell migration specifically on collagen by increasing cell adhesive ability through specific gene and protein upregulation of the β1 integrin subunit. Our results suggest that this naturally occurring green tea polyphenol may thus be used as a nutraceutical therapeutic agent in targeting the invasive character of medulloblastomas. J. Cell. Biochem. 90: 745–755, 2003.

Key words: cell adhesion; migration; integrin; EGCG; green tea; medulloblastoma

Brain tumors are the second leading cause of cancer in children. Among these tumors, the highly metastatic medulloblastomas represent approximately 25% of all pediatric intracranial neoplasms [Saran, 2002]. These neoplasms arise from the cerebellum and mainly affect children between ages 5 and 15 [Weil, 2002]. Medulloblastomas are currently treated by surgical intervention, generally combined with aggressive chemotherapy [Kaaijk et al., 2003; Mazzola and Pollack, 2003]. However, the 6-year survival rate of children with medulloblastoma still remains at only 14–17% [Hage et al., 2002]. Consequently, new clinical approaches need to be developed in order to improve the efficiency of current treatments.

Nutraceutical approaches have recently been the focus of attempts to treat several diseases such as osteoarthritis [Moreau et al., 2003], asthma [Hosseini et al., 2001], age-related diseases [Villeponteau et al., 2000], and cancer [Park and Pezzuto, 2002]. Among these nutraceutical substances, green tea has been lauded for various beneficial health effects [Sato and Miyata, 2000]. It has also been shown that intake of green tea polyphenols inversely correlated with cardiovascular mortality, hypertension, and diabetes [Dufresne and Farnworth, 2001]. Moreover, green tea can also modulate the invasive properties of numerous tumor cells

Abbreviations used: EGCG, (−)epigallocatechin-3-gallate; EGC, (−)epigallocatechin; ECM, extracellular matrix; MMP, matrix metalloproteinases.

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and prevent cancer [Kuroda and Hara, 1999; Mukhtar and Ahmad, 1999; Demeule et al., 2002]. Although the invasiveness of tumor cells strongly depends on their ability to migrate and to adhere to their extracellular matrix (ECM) environment, little is known about the molecular processes involved in brain cancer cell adhesion/migration or their modulation by green tea.

Cell adhesion is primarily mediated by a particular class of proteins called integrins. These are cell surface αβ-heterodimeric glycoproteins of which, to date, 24 αβ heterodimers formed by eight different β and 18 α subunits are known [Brakebusch et al., 2002]. Integrins bind to various ECM proteins such as collagen, fibronectin, laminin, and vitronectin, and their binding activates important intracellular signaling pathways that regulate, among other events, cell proliferation, and cell migration [Juliano, 2002]. Their functions have been implicated in processes such as development, the immune response, hemostasis, and maintenance of tissue integrity. Dysregulation of integrins functions is reflected in various pathological processes by chronic inflammation, tumor invasion, and metastasis [Hynes, 1992; Hemler, 1998]. Because integrins play a central role in tumor metastasis and tumor angiogenesis, they may thus represent crucial, specific targets for anti-tumoral treatments.

Green tea catechins, and in particular epigallocatechin-3-gallate (EGCG), have been demonstrated to reach the brain after oral intake [Nakagawa and Miyazawa, 1997; Suganuma et al., 1998]. One may thus assume that brain tumors such as medulloblastomas may be affected by the anti-tumoral activities of EGCG [Demeule et al., 2002]. In the present work, we demonstrate that EGCG can affect in vitro DAOY medulloblastoma cell adhesion/migration properties, and that these modulations are, in part, attributed to a differential expression of specific cell surface integrins, notably β1 integrin. These findings suggest that EGCG could act as an efficient anti-tumoral agent by regulating DAOY medulloblastoma cell adhesion properties.

MATERIALS AND METHODS

Reagents

Modified Eagle's medium (MEM), TRIzol reagent, trypsin, penicillin, and streptomycin were purchased from Invitrogen (Burlington, Ont.). Fetal bovine serum (FBS) was from HyClone Laboratories (Logan, UT). Fibronectin was from Roche (Mississauga, Ont.). Type I collagen was extracted from rat tail tendon according to standard protocols [Silver and Trelstad, 1980]. Vitronectin was prepared as previously described [Yatohgo et al., 1988]. EDTA, (−)epigallocatechin (EGC), EGCG, agarose, laminin-1, sodium dodecyl-sulfate (SDS), bovine serum albumin (BSA), and etoposide were purchased from Sigma (Oakville, Ont.).

Antibodies

Rat mAb 69.6.5 against αv integrin was produced as previously described [Lehmann et al., 1994]. Mouse mAbs Gi9 (anti-α2), C3VLA3 (anti-α3), HP2/1 (anti-α4), K20 (anti-β1), SZ21 (anti-β3), and rat mAb GoH3 (anti-α6) were from Immunotech (Marseille, France). Mouse mAbs FB12 (anti-α1), P1D6 (anti-α5), and LM609 (anti-αvβ3) were from Chemicon (Temecula, CA). The mouse mAb CSβ6 (anti-β6) was kindly provided by Dean Sheppard (University of California, San Francisco, CA). Goat anti-mouse FITC-conjugated antibodies were obtained from Jackson Immunoresearch (West Grove, PA). Sheep anti-rat FITC-conjugated antibodies were from Sigma (St. Louis, MO).

Cells and Culture Medium

The human DAOY medulloblastoma cell line was purchased from ATCC and was maintained in MEM containing 10% (v/v) FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin. Cells were incubated at 37°C, with 95% air and 5% CO_2. Subconfluent cells were used for all experiments. All treatments were performed in serum-free MEM for 18 h.

Cell Adhesion Assays

Adhesion assays were performed as previously described [Rigot et al., 1998]. Briefly, adhesion wells were coated with purified ECM protein solutions for 2 h at 37°C, then blocked by adding a solution of PBS/BSA 0.5%. Cells were harvested as a single cell suspension by treatment with 0.53 mM EDTA in PBS pH 7.2, added to precoated wells and allowed to adhere to the substrata for 2 h at 37°C. After washing, adherent cells were stained with a solution of 0.1% crystal violet/20% (v/v) methanol and lysed with 1% SDS. Spectrophotometric absorbance was then measured at 600 nm.

Migration Assays

The lower surface of Transwell filter inserts (8 μm pore size; Costar, Acton, MA) was precoated with type I collagen, laminin-1, vitronectin, or fibronectin, at a concentration of 10 μg/ml, for 2 h at 37°C. The Transwells were then assembled in a 24-well plate (Falcon, BD Biosciences, Mississaoga, ON), the lower chambers were filled with 600 μl of serum-free medium, and 100 μl of DAOY medulloblastoma cells (5×10^5 cells/ml) were inoculated into the upper chamber of each transwell. The plate was then left at 37°C in 95% air and 5% CO_2 for 2 h. Cells that had migrated to the lower surface of the filters were fixed, stained with 0.1% crystal violet/20% (v/v) methanol, and counted. Five random microscopic fields were counted at a magnification of ×200.

Flow Cytometry Analysis

Cell surface expression of integrin subunits on DAOY cells was measured by flow cytometry [Rigot et al., 1998]. Subconfluent cells were harvested and resuspended in MEM containing 20% FBS and 1% BSA. The single cell suspension (10^6 cells/ml) was incubated for 1 h at 4°C in the presence of anti-integrin mAbs (10 μg/ml each). Cells were rinsed once and then incubated in the same buffer with an appropriate secondary FITC-conjugated antibody for 1 h at 4°C. After washing, cells were fixed with 1% paraformaldehyde and cell-bound fluorescence was quantified using a Becton-Dickinson FACScan flow cytometer. To standardize our experimental conditions, all samples were prepared with the same cell suspension.

Fluorimetric Caspase-3 Assay

Cells which had been treated with either 50 μM etoposide, 10 or 20 μM of EGC or EGCG were collected and washed in cold PBS. Cells were lysed in Apo-Alert lysis buffer (Clontech, Palo Alto, CA) for 20 min at 4°C, and the lysates were further clarified by centrifugation at 16,000*g* for 20 min. Caspase-3 activity was determined by incubation with 50 μM caspase-3 specific fluorogenic peptide substrate acetyl-Asp-Glu-Val-Asp-7-AFC (Ac-DEVD-AFC) in 50 mM HEPES pH 7.4, 100 mM NaCl, 10% sucrose, 0.1% 3-[(3-cholamidopropyl)dimethylammonio-1-propane-sulfonate], 5 mM DTT, and 1 mM EDTA (assay buffer) on 96-well plates. The release of AFC ($\lambda_{ex} = 400$ nm, $\lambda_{em} = 505$ nm) was monitored for at least 20 min at 37°C on a SpectraMAX Gemini fluorescence plate reader (Molecular Devices, Sunnyvale, CA).

Total RNA Isolation and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from cultured DAOY medulloblastoma cells using the TRIzol Reagent. RT-PCR reactions were performed using the SuperScript™ One-Step RT-PCR with Platinum® Taq Kit from Invitrogen. RT-PCR reactions were performed with specific oligonucleotide primers derived from human sequences [Bell et al., 2001], and PCR conditions were optimized so that the gene products were obtained during the exponential phase of the amplification. PCR products were resolved on 1% (w/v) agarose gels containing 1 μg/ml ethidium bromide.

RESULTS

EGCG Specifically Decreases DAOY Cell Migration on Collagen

We previously demonstrated the capability of EGCG to inhibit several key processes involved in the invasive properties of brain tumor-derived U-87 glioblastoma cells [Annabi et al., 2002]. These included inhibition of soluble proMMP-2 and inhibition of MT1-matrix metalloproteinases (MMP) mediated cell invasion. Consequently, we sought to learn whether EGCG could also affect the migratory potential of medulloblastoma DAOY cells, a highly invasive pediatric brain tumor-derived cell line. We first determined the ECM specificity and basal migratory potential of DAOY cells using modified Boyden chambers with porous filters coated with purified ECM proteins. We found that DAOY cells displayed a stronger migratory potential towards collagen and vitronectin than to any other ECM protein, with laminin being the matrix protein towards which DAOY migrated the least (Fig. 1A). EGCG treatment of DAOY cells specifically inhibited cell migration on collagen in a dose-dependent manner, while no significant effect was observed on the other three substrates tested (Fig. 1B). A dose of 10 μM was found to inhibit approximately by half DAOY cell migration. Furthermore, EGC, a catechin that we previously reported not to affect U-87 glioma cell migration [Annabi et al.,

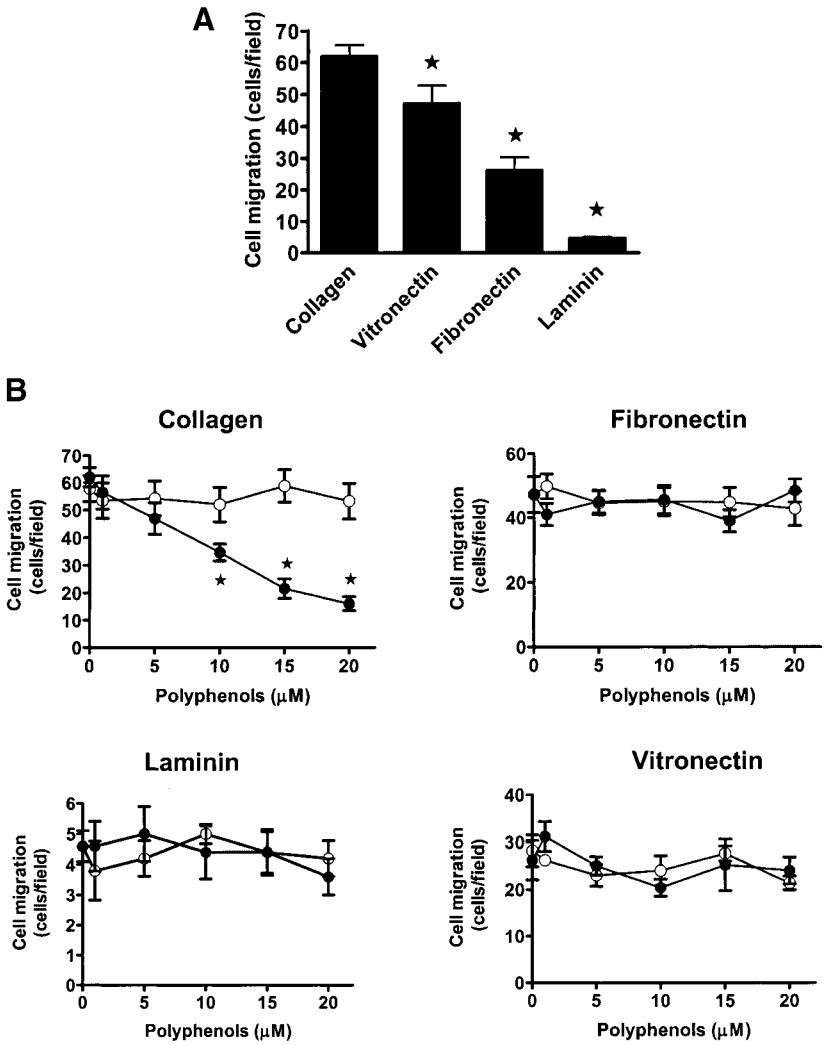

Fig. 1. Green tea catechin epigallocatechin-3-gallate (EGCG) specifically inhibits DAOY cell migration on collagen. **A**: Haptotaxis assays were performed in a transwell chamber, the lower surface of which was coated with either type I collagen, fibronectin, vitronectin, or laminin-1 at 10 μg/ml. **B**: DAOY cells were treated (or not) with increasing concentrations of EGCG (●) or (−)epigallocatechin (EGC, ○) for 18 h. Single cell suspension (5×10^4) were seeded onto the upper surface of transwells and allowed to migrate for 2 h. Cells that migrated to the bottom surface were stained as described in Materials and Methods. Data are presented as the mean number (±SEM) of cells migrated per field of microscope (magnification ×200) from at least three independent experiments. ★, Indicates statistically significant differences ($P < 0.05$) compared with the EGC treatment of DAOY using Student's *t*-test.

2002] did not affect DAOY migration on any of the ECM substrates tested. We conclude that EGCG specifically inhibits DAOY cell migration on collagen and that the gallate moiety of EGCG may, in part, be a crucial structural feature involved in this process and that should be further investigated.

DAOY Cell Adhesion on Collagen Is Increased by EGCG Treatment

Cell migration is intimately associated with cell adhesive properties. We thus assessed the effects of EGCG and EGC on DAOY cell adhesion. As treatment with 10 μM EGCG significantly reduced DAOY cell migration, we used this concentration of polyphenol for further experiments. We performed adhesion assays with various concentrations of different purified ECM proteins. As shown in Figure 2, DAOY cells adhered more readily to collagen and vitronectin than to fibronectin or laminin. Half-maximal binding was achieved at 0.25 μg/ml for collagen and 0.35 μg/ml for vitronectin, while the corresponding values for laminin

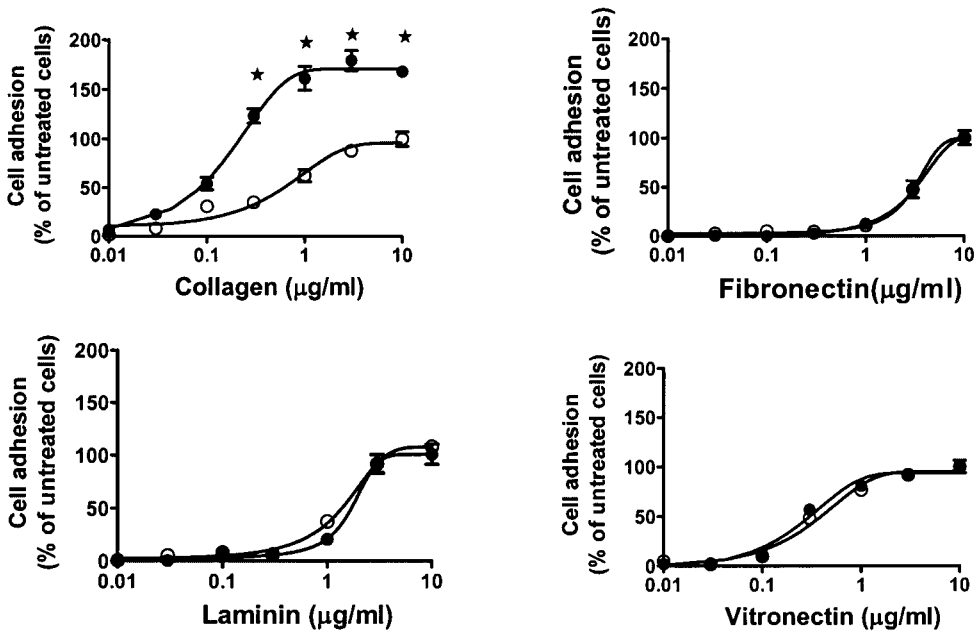

Fig. 2. Green tea catechin EGCG specifically increases DAOY cell adhesion to collagen. DAOY cells were treated as described in the legend to Figure 1 for 18 h with either carrier (○) or 10 μM EGCG (●). The cells were harvested with a 0.53 mM EDTA/PBS solution and washed twice with adhesion buffer. Cells (5×10^4) were seeded on a 96-well plate, previously coated with various purified extracellular matrix (ECM) proteins. Cells were allowed to adhere for 2 h at 37°C. Data shown are mean ± SEM of at least three independent experiments performed in triplicate.

and vitronectin are 2.0 and 2.5 μg/ml, respectively. EGCG treatment of DAOY increased cell adhesion and affinity to collagen, without significantly affecting cell adhesive properties on fibronectin, vitronectin, or laminin (Fig. 2). No effect of EGC treatment on DAOY cell adhesion was observed with collagen or the other matrices (not shown).

Green Tea Catechins EGCG and EGC do not Trigger DAOY Cell Apoptosis

When used above 10 μM, green tea polyphenols can trigger pro-apoptotic events in several cell types [Islam et al., 2000]. To address this issue, we measured the activation of caspase-3, the activity of which has emerged as a powerful marker for cells undergoing apoptosis [Abu-Qare and Abou-Donia, 2001]. As a positive control, cells were treated with etoposide, a pro-apoptotic agent and a strong inducer of caspase-3. As expected, etoposide strongly induced caspase-3 expression (6.3-fold), while neither EGCG nor EGC triggered, at the two concentrations used, any activation of caspase-3 (Fig. 3). Moreover, no necrosis was observed in EGC- or EGCG-treated DAOY cells when cell viability was tested by a trypan blue exclusion assay (not shown). This lack of pro-apoptotic effects was noticed in other cell lines such as U-87 glioma [Annabi et al., 2002], endothelial cells [Lamy et al., 2002], and mesenchymal stem

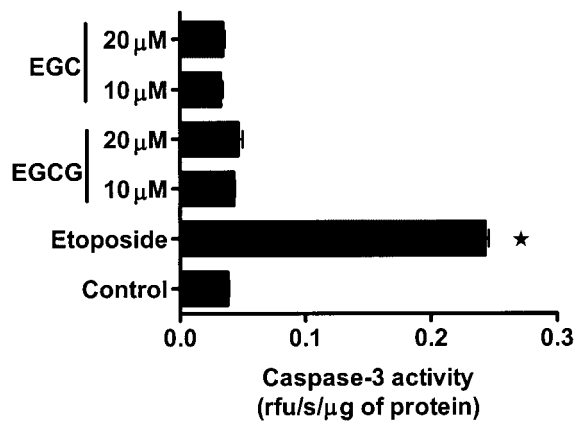

Fig. 3. Green tea catechins EGCG and EGC do not induce pro-apoptotic activity in DAOY cells. Fluorimetric caspase-3 activation assays have been performed using cells which were treated for 18 h with either 50 μM etoposide, 10 and 20 μM EGCG or EGC. ★, Indicates statistically significant differences ($P < 0.05$) compared with control (untreated DAOY) cells using Student's *t*-test.

cells [Annabi et al., 2003a], but does not preclude that viability of other cells be as sensitive to EGCG. This crucial observation further emphasizes the efficacy and immediate safe use of low green tea catechin concentrations in future clinical treatments.

β1 Integrin Subunit Mediates DAOY Cell Adhesion to Collagen

The integrins are the best characterized cell surface adhesion receptors. These transmembrane proteins play a crucial role in several steps in cell motility, including cell adhesion and migration. Because EGCG can modulate these two cellular processes, we first characterized the integrins present at the DAOY cell surface using flow cytometry. A broad spectrum of integrin subunits was expressed at the DAOY cell surface. Fluorescence data clearly show that integrin αvβ3, α2, α3, α4, α6, αv, β1, and β3 subunits were expressed in DAOY cells, while levels of α5 and β6 subunit expression were very low (Fig. 4A). Such spectrum reflects the many cellular processes that may be tightly regulated by these integrins and which include: growth, death (apoptosis), adhesion, migration, and invasion by activating several signaling pathways. These integrins are thus attractive targets for anti-tumoral drug design aimed at inhibiting integrin activity, which may thus be useful in controlling the metastatic spread of cancer.

Since EGCG only affected cell migration and adhesion on collagen, we hypothesized that this catechin could modulate the expression of the specific integrin subunits responsible for cell adhesion on that particular matrix. Since various integrin subunits can interact with collagen, we tried to determine which were involved in the collagen adhesion process in DAOY. We thus performed experiments with specific integrin blocking antibodies. We incubated DAOY cells only with the antibodies directed towards the four main integrin subunits known to interact with collagen (i.e., α1, α2, α3, and β1 integrin) and performed adhesion assays on collagen. While functional blocking of α1 and α3 integrins did not affect DAOY cell adhesion to collagen, blocking of α2 subunits lead to a 40% decrease in cell adhesion to collagen (Fig. 4B). Moreover, when DAOY were incubated in the presence of anti-β1 antibody, cell adhesion to collagen was almost completely antagonized (Fig. 4B). This strongly suggests that β1-containing integrins, including the integrin α2β1, are the major receptors mediating DAOY cell adhesion to collagen.

EGCG Upregulates the Gene and Cell Surface Expression of the β1 Integrin Subunit in DAOY Cells

Flow cytometry analysis was performed in order to investigate the effect of EGCG treatment on cell surface integrin expression. Once again, only α1, α2, α3, and β1 subunit expressions were investigated because these integrins are known to mediate cell adhesion on collagen in many cell types. As a negative control, we also monitored the expression of β3 subunit, which does not interact with collagen. We found that α1, α2, and α3 integrin expression were not altered by treatment of DAOY cells with EGCG and that no variation in the expression of β3 subunit was observed (Fig. 5A). However, we observed a strong increase ($\approx 50\%$) in β1 subunit expression which was induced by EGCG, as demonstrated by the shift in fluorescence intensity. This increase in β1 integrin at the cell surface of EGCG-treated DAOY cells was corroborated by the demonstration of an increase in β1 integrin transcript levels using RT-PCR, while α2 and β3 integrin transcripts remained unaffected (Fig. 5B).

DISCUSSION

Medulloblastomas represent one of the most aggressive pediatric brain tumors. Currently, no treatment other than surgery exists to definitively prevent tumor development. The development of new therapeutic approaches, such as those aimed at targeting tumor neovascularization, is obviously needed. Among the anti-angiogenic agents developed and tested in the last few years, the anti-cancer properties of naturally occurring green tea polyphenols have shown promising potential to inhibit tumor growth and to target the invasive characteristics of cancer cells [Demeule et al., 2002]. Although the effects of EGCG, the most abundant catechin and also the main active component of green tea, on cell migration/adhesion have been recently investigated [Zhang et al., 2000; Suzuki and Isemura, 2001], no studies have examined the effect of EGCG on the modulation of the specific cell adhesion receptors involved. We thus tested the effect of EGCG on several motility processes of DAOY

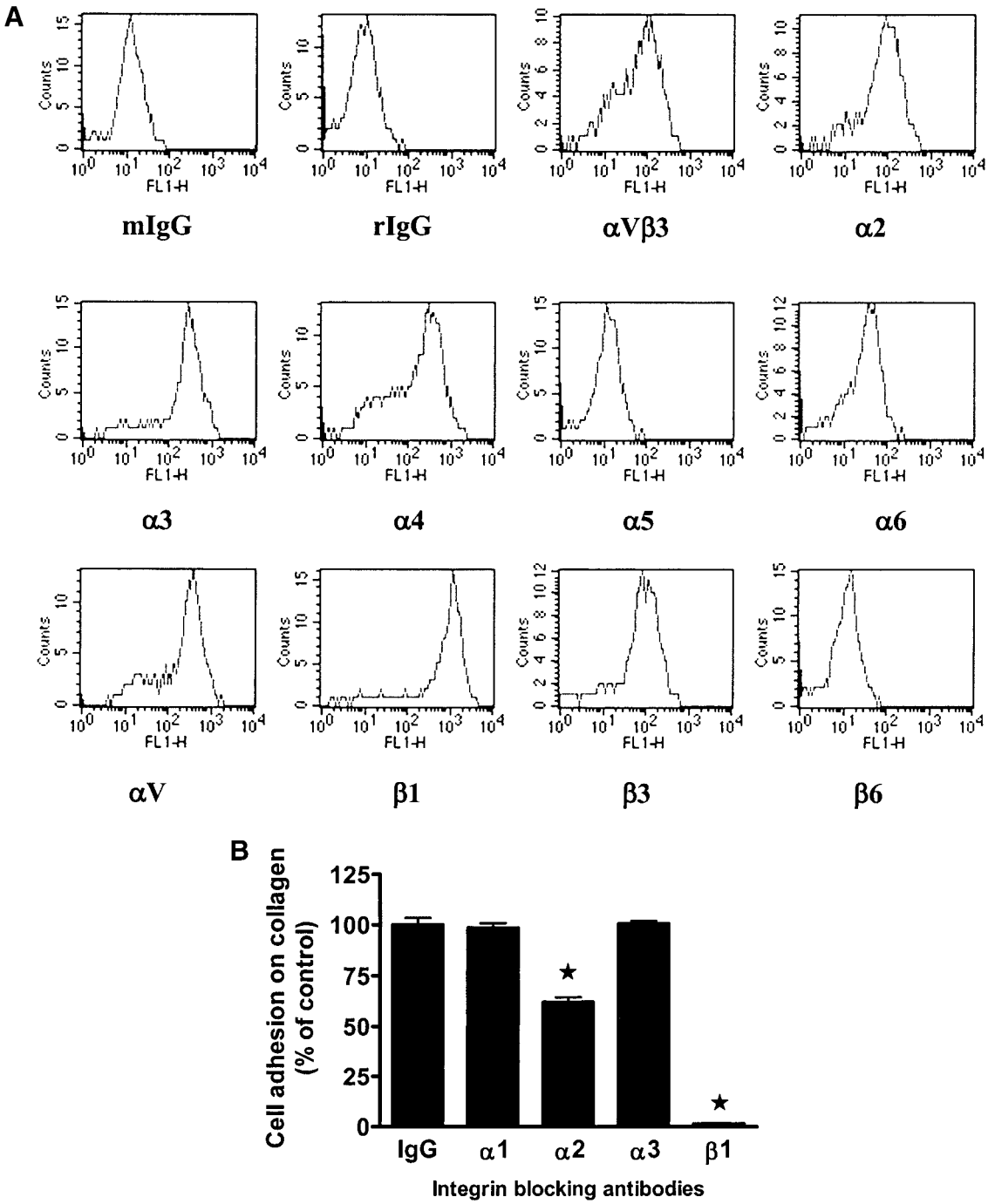

Fig. 4. Expression of integrin subunits at the DAOY cell surface. **A**: Flow cytometric analysis was performed using antibodies directed towards specific integrin subunits as described in Materials and Methods. **B**: Adhesion assays have been performed with DAOY cells preincubated with blocking antibodies directed against various integrin subunits. Cells were allowed to adhere on 10 μg/ml collagen for 2 h at 37°C. All assays were performed in triplicate and the results (±SEM) of three independent experiments are shown.

medulloblastoma cells. We demonstrate that EGCG affected both DAOY in vitro cell migration and ECM adhesion properties.

Specifically, we show that DAOY cells express high levels of the β1 integrin subunit and that attachment of DAOY cells to type-I collagen is completely abolished by function-blocking antibodies against the β1 integrin subunit. We also demonstrate that EGCG treatment specifically upregulated both the gene and protein

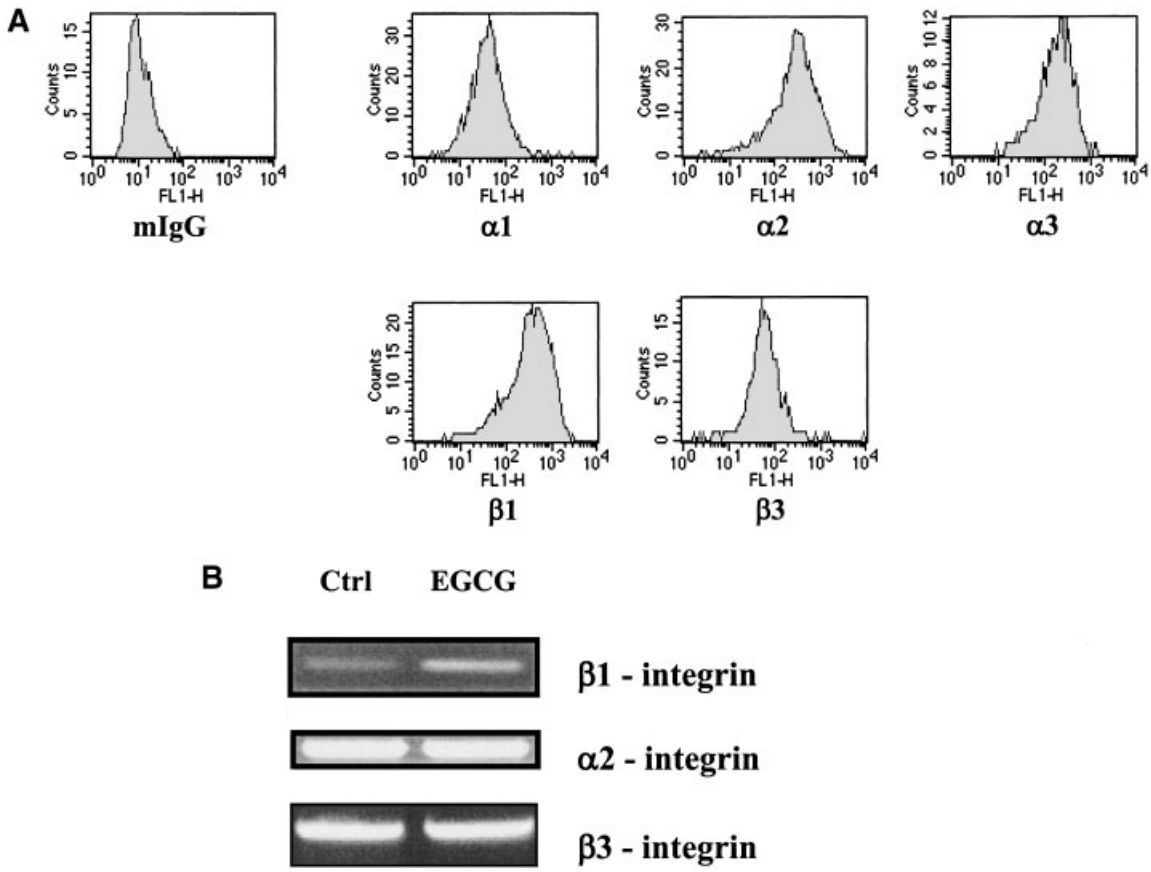

Fig. 5. Green tea catechin upregulates β1 integrin subunit expression at the DAOY cell surface. DAOY cells were treated (or not) with 10 μM EGCG for 18 h. **A**: Expression of α1, α2, α3, β1, and β3 at the cell surface was monitored by flow cytometry as described in Materials and Methods (shaded area: control; lined curve: EGCG treatment). **B**: Total RNA was isolated from untreated or EGCG-treated DAOY cells and β1, α2, and β3 integrin subunits gene products were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR).

expression of β1 integrin subunit in DAOY cells. Our study thus provides cellular and molecular evidence that cell surface integrins may represent a novel molecular target for green tea catechin EGCG in DAOY cells. Further in vivo experimentation will have to be required in order to demonstrate EGCG's efficacy as an anti-tumor agent in patients afflicted with pediatric brain tumors.

Since cell migration can be considered as a finely regulated equilibrium between adhesion and de-adhesion of cells [Friedl and Brocker, 2000], a differential expression of cell adhesion receptors induced by EGCG may thus impact on DAOY cell migration. Such an effect of cell surface integrin expression on cell motility has been suggested [Palecek et al., 1997], and a strict correlation can thus be established between increased adhesion and decreased cell migration in DAOY cells upon EGCG treatment. One can thus safely postulate that the inhibition of DAOY cell migration can be explained by the upregulation of the β1 integrin subunit, which would favor the immobilization of DAOY cells on type-I collagen.

Integrin receptors involved in collagen binding such as the β1 integrin are known to associate with different α subunits. It is tempting to suggest that the functionality of heterodimers that could form through the association with specific α integrin subunits could also be modulated by EGCG in DAOY cells. Since only adhesion to collagen was affected by EGCG, and because α1, α2, and α3 integrin subunit expression was not modulated, we cannot exclude the possibility that the expression of other α integrin subunits such as α10 and α11, both reputed to interact with collagen, could be modulated by EGCG. Moreover, in addition to cell surface expression, integrin adhesive functions can be regulated by changes either in the affinity of the individual receptors for their

respective ligands or in integrin avidity (i.e., integrin clustering) which may allow more efficient interaction between cells and ECM [Gonzalez-Amaro and Sanchez-Madrid, 1999]. Therefore, although EGCG treatment of DAOY cells resulted in the specific upregulation of the β1 integrin subunit, an increase of affinity or avidity in association with other α integrin subunits cannot be excluded.

Many of the documented effects of EGCG treatments on cell migration have mainly been correlated to the inhibitory effect of EGCG on MMP, a major class of proteolytic enzymes also actively implicated in ECM remodeling and which play a crucial role in the cell migration process [Annabi et al., 2002; Dell'Aica et al., 2002; Islam et al., 2002]. Thus, the inhibition of DAOY cell migration that we report in the present study may not be solely attributed to an upregulation of collagen adhesion receptors. MMPs participate in the invasion process and, consequently, are highly involved in cell migration [Visse and Nagase, 2003]. Among them, the MMP-2 and MMP-9 collagenases have been shown to display a strong pro-migratory activity [Hornebeck et al., 2002]. We have already demonstrated that EGCG inhibited proMMP-2 secretion in U-87 glioblastoma cells [Annabi et al., 2002]. Interestingly, we also observed that proMMP-2 secretion was partially inhibited by EGCG treatment in medulloblastoma-derived DAOY cells (unpublished data). We thus cannot exclude a potential role of EGCG-mediated inhibition of MMP-2 secretion in the modulation of DAOY cells motility processes.

Although adhesion is the primary function of integrins, they are also important signaling molecules. Integrins regulate intracellular signaling pathways controlling cell migration and invasion, cytoskeletal organization, force generation, and survival [Giancotti and Ruoslahti, 1999; Parise et al., 2000]. Whether EGCG interferes with any of the intracellular integrin-mediated signaling in DAOY cells is currently under investigation and still remains to be established. It is worth noting that recent evidence suggests that the PI3K/Akt pathway, a crucial integrin-mediated signaling pathway involved in apoptosis, is affected by EGCG [Waltner-Law et al., 2002]. This pathway particularly involves the functions of an integrin-linked kinase (ILK) which promotes cell proliferation through the activation of PKB/Akt anti-apoptotic signaling pathway [Cruet-Hennequart et al., 2003; Troussard et al., 2003]. Since integrins have no intrinsic enzymatic activity, it will be crucial to assess whether other integrin-associated proteins, such as the src-family kinases (c-src, fyn, yes), FAK, PKA, and Pyk-2 [Miranti and Brugge, 2002], may also be targeted by EGCG. Ultimately, EGCG may also have a dual role in targeting both medulloblastoma-derived cancer and endothelial cells. Interestingly, β1 integrins, which are not unique to angiogenic endothelial cells, may also support most of the adhesion, migration, and signaling events essential for angiogenesis [Luscinskas and Lawler, 1994]. Current studies in our laboratory are underway to explore the above transduction pathways that may be modulated by EGCG. Moreover, special focus will be directed towards the identification of the β1 integrin(s) mediating endothelial/cancer cell adhesion, migration, and signaling events required for angiogenesis and metastasis.

Suppressing tumor growth by anti-angiogenesis and vascular targeting strategies has led to the recent development of a large number of natural and synthetic anti-angiogenic compounds [Ranieri and Gasparini, 2001]. These include inhibitors of VEGF, VEGF-R2, MMP, and integrin antagonists. Experimental evidence suggests that anti-angiogenic treatments may be more effective in suppressing tumor growth when used in combination with conventional chemotherapy, immunotherapy, or radiotherapy [Koukourakis, 2001]. Interestingly, recent evidence from our laboratory has demonstrated the pleitropic effects of green tea catechin EGCG on MMP-mediated events in glioblastoma cells [Annabi et al., 2002], and on suppressing tubulogenesis and VEGF-R2 receptor mediated effects in endothelial cells [Lamy et al., 2002]. Moreover, we have shown that treatment of human umbilical vein endothelial cells (HUVEC) with doses of EGCG equivalent to those found in human plasma after oral administration (2–5 μM), combined with low-doses of ionizing radiation, affected the cell adhesive properties of HUVEC and suppressed tubulogenesis [Annabi et al., 2003b]. In light of the data obtained in the present study, it is thus tempting to suggest that low-dose metronomic administration of naturally occurring green tea polyphenol may provide, in combination with radiotherapeutic modalities, a new perspective in treating pediatric patients with medulloblastoma.

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REFERENCES

Abu-Qare AW, Abou-Donia MB. 2001. Biomarkers of apoptosis: Release of cytochrome c, activation of caspase-3, induction of 8-hydroxy-2′-deoxyguanosine, increased 3-nitrotyrosine, and alteration of *p53* gene. J Toxicol Environ Health B Crit Rev 4:313–332.

Annabi B, Lachambre MP, Bousquet-Gagnon N, Pagé M, Gingras D, Béliveau R. 2002. Green tea polyphenol (−)-epigallocatechin 3-gallate inhibits MMP-2 secretion and MT1-MMP-driven migration in glioblastoma cells. Biochim Biophys Acta 1542:209–220.

Annabi B, Lee YT, Turcotte S, Naud E, Desrosiers RR, Champagne M, Eliopoulos N, Galipeau J, Béliveau R. 2003a. Hypoxia promotes bone marrow-derived stromal cell migration and tube formation. Stem Cells 21:337–347.

Annabi B, Lee YT, Martel C, Pilorget A, Bahary JP, Béliveau R. 2003b. Radiation induced-tubulogenesis in endothelial cells is antagonized by the antiangiogiogenic properties of green tea polypher (−) epigallocatechin−3−gallate. Cancer Biol Ther 2:1–8.

Bell SE, Mavila A, Salazar R, Bayless KJ, Kanagala S, Maxwell SA, Davis GE. 2001. Differential gene expression during capillary morphogenesis in 3D collagen matrices: Regulated expression of genes involved in basement membrane matrix assembly, cell cycle progression, cellular differentiation, and G-protein signaling. J Cell Sci 114:2755–2773.

Brakebusch C, Bouvard D, Stanchi F, Sakai T, Fassler R. 2002. Integrins in invasive growth. J Clin Invest 109: 999–1006.

Cruet-Hennequart S, Maubant S, Luis J, Gauduchon P, Staedel C, Dedhar S. 2003. Alpha(v) integrins regulate cell proliferation through integrin-linked kinase (ILK) in ovarian cancer cells. Oncogene 22:1688–1702.

Dell'Aica I, Dona M, Sartor L, Pezzato E, Garbisa S. 2002. (−)Epigallocatechin-3-gallate directly inhibits MT1-MMP activity, leading to accumulation of nonactivated MMP-2 at the cell surface. Lab Invest 82:1685–1693.

Demeule M, Michaud-Levesque J, Annabi B, Gingras D, Boivin D, Jodoin J, Lamy S, Bertrand Y, Béliveau R. 2002. Green tea catechins as novel antitumor and antiangiogenic compounds. Curr Med Chem Anticancer Agents 2:441–463.

Dufresne CJ, Farnworth ER. 2001. A review of latest research findings on the health promotion properties of tea. J Nutr Biochem 12:404–421.

Friedl P, Brocker EB. 2000. The biology of cell locomotion within three-dimensional extracellular matrix. Cell Mol Life Sci 57:41–64.

Giancotti FG, Ruoslahti E. 1999. Integrin signaling. Science 285:1028–1032.

Gonzalez-Amaro R, Sanchez-Madrid F. 1999. Cell adhesion molecules: Selectins and integrins. Crit Rev Immunol 19:389–429.

Hage R, Duurkens VA, Seldenrijk CA, Brutel de la Riviere A, van Swieten HA, van den Bosch JM. 2002. Primitive neuroectodermal tumor: Report of two cases and review of the literature. J Thorac Cardiovasc Surg 124:833–836.

Hemler ME. 1998. Integrin associated proteins. Curr Opin Cell Biol 10:578–585.

Hornebeck W, Emonard H, Monboisse JC, Bellon G. 2002. Matrix-directed regulation of pericellular proteolysis and tumor progression. Semin Cancer Biol 12:231–241.

Hosseini S, Pishnamazi S, Sadrzadeh SM, Farid F, Farid R, Watson RR. 2001. Pycnogenol(R) in the management of asthma. J Med Food 4:201–209.

Hynes RO. 1992. Integrins: Versatility, modulation, and signaling in cell adhesion. Cell 69:11–25.

Islam S, Islam N, Kermode T, Johnstone B, Mukhtar H, Moskowitz RW, Goldberg VM, Malemud CJ, Haqqi TM. 2000. Involvement of caspase-3 in epigallocatechin-3-gallate-mediated apoptosis of human chondrosarcoma cells. Biochem Biophys Res Commun 270:793–797.

Islam S, Islam N, Kermode T, Johnstone B, Mukhtar H, Moskowitz RW, Goldberg VM, Malemud CJ, Haqqi TM. 2002. (−)Epigallocatechin-3-gallate directly inhibits MT1-MMP activity, leading to accumulation of nonactivated MMP-2 at the cell surface. Lab Invest 82:1685–1693.

Juliano RL. 2002. Signal transduction by cell adhesion receptors and the cytoskeleton: Functions of integrins, cadherins, selectins, and immunoglobulin-superfamily members. Annu Rev Pharmacol Toxicol 42:283–323.

Kaaijk P, Schouten-van Meeteren AY, Slotman BJ, Kaspers GJ. 2003. Past, current, and future protocols for combined modality therapy in childhood medulloblastoma. Expert Rev Anticancer Ther 3:79–90.

Koukourakis MI. 2001. Tumour angiogenesis and response to radiotherapy. Anticancer Res 21:4285–4300.

Kuroda Y, Hara Y. 1999. Antimutagenic and anticarcinogenic activity of tea polyphenols. Mutat Res 436: 69–97.

Lamy S, Gingras D, Béliveau R. 2002. Green tea catechins inhibit vascular endothelial growth factor receptor phosphorylation. Cancer Res 62:381–385.

Lehmann M, Rabenandrasana C, Tamura R, Lissitzky JC, Quaranta V, Pichon J, Marvaldi JA. 1994. Monoclonal antibody inhibits adhesion to fibronectin and vitronectin of a colon carcinoma cell line and recognizes the integrins alpha v beta 3, alpha v beta 5, and alpha v beta 6. Cancer Res 54:2102–2107.

Luscinskas FW, Lawler J. 1994. Integrins as dynamic regulators of vascular function. FASEB J 8:929–938.

Mazzola CA, Pollack IF. 2003. Medulloblastoma. Curr Treat Options Neurol 5:189–198.

Miranti CK, Brugge JS. 2002. Sensing the environment: A historical perspective on integrin signal transduction. Nat Cell Biol 4:E83–E90.

Moreau M, Dupuis J, Bonneau NH, Desnoyers M. 2003. Clinical evaluation of a nutraceutical, carprofen, and meloxicam for the treatment of dogs with osteoarthritis. Vet Rec 152:323–329.

Mukhtar H, Ahmad N. 1999. Green tea in chemoprevention of cancer. Toxicol Sci 52:111–117.

Nakagawa K, Miyazawa T. 1997. Absorption and distribution of tea catechin, (−)-epigallocatechin-3-gallate, in the rat. J Nutr Sci Vitaminol (Tokyo) 43:679–684.

Palecek SP, Loftus JC, Ginsberg MH, Lauffenburger DA, Horwitz AF. 1997. Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. Nature 385:537–540.

Parise LV, Lee J, Juliano RL. 2000. New aspects of integrin signaling in cancer. Semin Cancer Biol 10:407–414.

Park EJ, Pezzuto JM. 2002. Botanicals in cancer chemoprevention. Cancer Metastasis Rev 21:231–255.

Ranieri G, Gasparini G. 2001. Angiogenesis and angiogenesis inhibitors: A new potential anticancer therapeutic strategy. Curr Drug Targets Immune Endocr Metabol Disord 1:241–253.

Rigot V, Lehmann M, Andre F, Daemi N, Marvaldi J, Luis J. 1998. Integrin ligation and PKC activation are required for migration of colon carcinoma cells. J Cell Sci 111:3119–3127.

Saran F. 2002. Recent advances in paediatric neuro-oncology. Curr Opin Neurol 15:671–677.

Sato T, Miyata G. 2000. The nutraceutical benefit, part I: Green tea. Nutrition 16:315–317.

Silver FH, Trelstad RL. 1980. Type I collagen in solution. Structure and properties of fibril fragments. J Biol Chem 255:9427–9433.

Suganuma M, Okabe S, Oniyama M, Tada Y, Ito H, Fujiki H. 1998. Wide distribution of [^{3}H](−)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. Carcinogenesis 19:1771–1776.

Suzuki Y, Isemura M. 2001. Inhibitory effect of epigallocatechin gallate on adhesion of murine melanoma cells to laminin. Cancer Lett 173:15–20.

Troussard AA, Mawji NN, Ong C, Mui A, St. Arnaud R, Dedhar S. 2003. Conditional knock-out of integrin-linked kinase (ILK) demonstrates an essential role in PKB/Akt activation. J Biol Chem 278:22374–22378.

Villeponteau B, Cockrell R, Feng J. 2000. Nutraceutical interventions may delay aging and the age-related diseases. Exp Gerontol 35:1405–1417.

Visse R, Nagase H. 2003. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. Circ Res 92:827–839.

Waltner-Law ME, Wang XL, Law BK, Hall RK, Nawano M, Granner DK. 2002. Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. J Biol Chem 277:34933–34940.

Weil MD. 2002. Primitive neuroectodermal tumors/medulloblastoma. Curr Neurol Neurosci Rep 2:205–209.

Yatohgo T, Izumi M, Kashiwagi H, Hayashi M. 1988. Novel purification of vitronectin from human plasma by heparin affinity chromatography. Cell Struct Funct 13:281–292.

Zhang G, Miura Y, Yagasaki K. 2000. Suppression of adhesion and invasion of hepatoma cells in culture by tea compounds through antioxidative activity. Cancer Lett 159:169–173.