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Antitumor activity of the tea polyphenol epigallocatechin-3-gallate encapsulated in targeted vesicles after intravenous administration

Aim: The therapeutic potential of epigallocatechin-3-gallate (EGCG), a green tea polyphenol with anticancer properties, is limited by its inability to specifically reach tumors following intravenous administration. The purpose of this study was to determine whether a tumor-targeted vesicular formulation of EGCG would suppress the growth of A431 epidermoid carcinoma and B16-F10 melanoma in vitro and in vivo. Materials & methods: Transferrin-bearing vesicles encapsulating EGCG were administered intravenously to mice bearing subcutaneous A431 and B16-F10 tumors. Results: The intravenous administration of EGCG encapsulated in transferrin-bearing vesicles resulted in tumor suppression in 40% of A431 and B16-F10 tumors. Animal survival was improved by more than 20 days compared with controls. Conclusion: Encapsulation of EGCG in transferrin-bearing vesicles is a promising therapeutic strategy.

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KEYWORDS: cancer therapy epigallocatechin-3-gallate green tea transferrin tumor targeting

Epigallocatechin-3-gallate (EGCG), the most abundant polyphenol in green tea, has been extensively studied for its cancer chemopreventive and therapeutic potential against various cancer types [1,2]. It has been shown to be a potent inducer of cell death and cell-cycle arrest in various cancer types in vitro and in vivo via different signaling pathways and mechanisms. Previous studies demonstrated that it exerts its anticancer effect by modulating the activity of MAPK, death-associated protein kinase 2, IGF receptor, Akt kinase and NF-κB. EGCG has also been shown to induce apoptosis via inhibition of VEGF signaling and production of hydrogen peroxide [2]. However, the inability of this polyphenol to specifically reach tumors following intravenous administration limits its potential therapeutic use. Furthermore, as a result of its poor oral bioavailability, only 0.1–1.1% of the orally administered EGCG dose is able to reach the systemic circulation, leading to plasma EGCG concentrations that are five- to 50-times lower than those shown to exert biological activities in vitro [3]. Given the potent antiproliferative properties of EGCG, it is highly important to target the delivery of this polyphenol specifically to its site of action.

To remediate this problem, the authors proposed to encapsulate EGCG in transferrin (Tf)-bearing vesicles. These vesicles are particularly attractive for selective receptor-mediated drug delivery to tumors because of their ability to recognize Tf receptors that are overexpressed on many types of cancer cells [4,5]. The combination of Tf ligands with the passive accumulation of particulate delivery systems in tumors due to enhanced permeability and retention has been widely used in tumor-targeted strategies [6,7–12]. The objectives of this study were, therefore, to prepare and characterize Tf-bearing vesicles encapsulating EGCG and to evaluate their therapeutic efficacy in vitro and in vivo following intravenous administration.

Materials & methods

Cell lines & reagents

EGCG, iron-saturated human Tf and all other chemicals were purchased from Sigma–Aldrich (Poole, UK). Solulan™ C24 was obtained from Amerchol™ (NJ, USA). DMEM, RPMI-1640 media, fetal bovine serum, l-glutamine and penicillin-streptomycin was obtained from Invitrogen™ (Paisley, UK). Dialysis tubing (molecular weight cutoff: 1350 Da) was obtained from Medicell International (London, UK). A431 human epidermoid carcinoma and T98G human glioblastoma were purchased from the European Collection of Cell Cultures (Salisbury, UK). d-Luciferin and Bioware® B16-F10-luc-G5 mouse melanoma were obtained from Caliper Life Sciences (MA, USA).
Preparation & characterization of Tf-bearing vesicles encapsulating EGCG

Span™ 60 (65 mg), cholesterol (58 mg) and Solulan™ C24 (54 mg) were shaken in 2 ml EGCG solution (1 mg/ml in glucose 5% solution) for 1 h at 60°C, followed by probe sonication (Soniprep 150, Fisher Scientific, Loughborough, UK) for 2 × 2 min, at 75% of the maximal capacity of the sonicator. TF (12 mg) was conjugated to these vesicles (2 ml) by using the crosslinker dimethylsulphodimide (24 mg), as previously described [7,8]. Unencapsulated EGCG was removed by ultracentrifugation, as previously described [7].

Vesicles were visualized by transmission electron microscopy on a LEO 912 energy-filtering electron microscope (Sigma–Aldrich). Briefly, Formvar/carbon-coated 200-mesh copper grids were glow discharged and specimens in distilled water were dried down with filter paper to a thin layer onto the hydrophilic support film. 20 µl of 1% aqueous methylamine vanadate stain (NanoVan®) was applied and the mixture dried down immediately using filter paper. Specimens were imaged with a LEO 912 energy filtering transmission electron microscope operating at 120 kV. Contrast-enhanced, zero-loss energy filtered digital images were recorded with a 14 bit/2K charge-coupled device camera.

The amount of EGCG encapsulated in the vesicles was quantified by spectrofluorimetry ($\lambda_{em}$: 305 nm; $\lambda_{ex}$: 345 nm), using a Varian Cary Eclipse spectrofluorometer (Agilent Technologies, CA, USA) following the disruption of vesicles with isopropanol. The amount of TF linked to vesicles was assessed using the Lowry method [7]. The size and zeta potential of the vesicles were determined through photon correlation spectroscopy and laser Doppler electrophoresis, using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK).

The release of EGCG from TF-bearing vesicles was determined by dialysis of 1 ml vesicles against 10 ml phosphate-buffered saline at 37°C, whilst stirring for 24 h. At different time-points, a 1 ml sample of the dialysate was withdrawn and immediately replaced with an equal volume of fresh solution. The amount of EGCG present in the samples was quantified by spectrofluorimetry ($\lambda_{ex}$: 305 nm; $\lambda_{em}$: 345 nm).

In vitro antiproliferative activity

A431, T98G and B16-F10 cells were seeded at a density of 2 × 10³ cells/well in 96-well plates and grown for 72 h prior to being treated with EGCG (10 µg/well) in solution or encapsulated in TF-bearing or control vesicles. After the 72-h treatment, the cells were washed three times with phosphate-buffered saline and lysed. The amount of EGCG in the cell lysate was quantified by spectrofluorimetry ($\lambda_{ex}$: 305 nm; $\lambda_{em}$: 345 nm).

The cellular uptake of EGCG encapsulated in TF-bearing vesicles was also qualitatively analyzed by coherent anti-Stokes Raman scattering microscopy [13]. This novel form of microscopy presents the advantage of selectively detecting molecules without using any exogenous label. A431, T98G and B16-F10 cells were seeded in four-well chamber slides (10⁴ cells/well) and grown at 37°C for 72 h. They were then treated with EGCG (10 µg/well), in solution or encapsulated in TF-bearing or control vesicles. After the 24-h treatment, the cells were then washed three times with phosphate-buffered saline, fixed in methanol for 10 min and rinsed with distilled water before being examined using a custom-built coherent anti-Stokes Raman scattering microscope and a previously described technique [10,13]. EGCG was detected at 2916 cm⁻¹.

In vivo tumoricidal activity

All animal experiments conducted were approved by local ethics committees and performed in accordance with the UK Home Office regulations. Female, nonfasting immunodeficient...
BALB/c mice (initial mean weight: 20 g) were housed in groups of five with a room temperature of 21 ± 2°C and alternating 12-h light–dark cycles. A431 or B16-F10-luc-G5 cancer cells in exponential growth were subcutaneously implanted to both flanks of the mice (1 × 10⁶ cells per flank). When tumors become palpable and reached a diameter of 5 mm, the mice were intravenously injected with EGCG encapsulated in Tf-bearing vesicles, control vesicles or in solution (10 μg EGCG per injection) once daily for 20 days. The mice were observed daily for tumor growth and changes in body weight. The tumor volume was determined by caliper measurements and calculated by the formula: volume = d³ × π/6. In accordance with the Response Evaluation Criteria in Solid Tumors [14], responses to treatment were classified as follows: progressive disease for relative tumor volume higher than 1.2-fold; stable disease for relative tumor volume between 0.7- and 1.2-fold; partial response for relative tumor volume between 0- and 0.7-fold; and complete response when the tumor has completely disappeared.

Tumor growth was also monitored by bioluminescence imaging with an IVIS® Spectrum (Caliper Life Sciences). Mice bearing subcutaneous B16-F10-luc-G5 tumors were treated intravenously as described earlier. In addition, 10 min prior to in vivo imaging, mice received the luciferase substrate d-luciferin (150 mg/kg bodyweight) by intraperitoneal injection and were anesthetized by isoflurane inhalation on days 1, 3, 5, 7 and 9. The light emitted from the bioluminescent tumors was detected for 2 min using Living Image® software (Caliper Life Sciences) and displayed as a pseudocolor overlay onto a grayscale animal image.

**Statistical analysis**

Results were expressed as mean ± standard error of the mean. Statistical significances were calculated with the one-way analysis of variance test followed by the Bonferroni multiple comparison post-test using the GraphPad Prism® software (GraphPad, CA, USA). P-values lower than 0.05 were considered statistically different.

**Results**

**Preparation & characterization of Tf-bearing vesicles encapsulating EGCG**

Vesicles encapsulating EGCG and conjugated to Tf were prepared. Transmission electron microscopy studies confirmed the spherical shape of these delivery systems (Figure 1A). Vesicles were able to encapsulate 30.3 ± 2.8% of the initial EGCG and were conjugated to 25.7 ± 2.5% of the initial Tf. As expected, the conjugation of Tf to the surface of vesicles resulted in a larger vesicular size (294 nm; polydispersity: 0.414) than that of control vesicles (205 nm; polydispersity: 0.427). These sizes were nevertheless below the cutoff size for extravasation, which was 400 nm for most tumors [15,16].

The grafting of Tf to the vesicles reduced their zeta potential compared with that of control vesicles (-36 and -28 mV, respectively). These net negative charges are actually advantageous for a delivery system compared with positively charged formulations, as they would reduce the electrostatic interactions of the vesicles with the negatively charged cell membrane and, therefore, should decrease the nonspecific uptake of the vesicles by the cells [17].

EGCG was released relatively readily from Tf-bearing vesicles (Figure 2): approximately 50% of the drug leaked out of the formulation 6 h after the start of the experiment. The release of the drug then reached a plateau from 10 h. The relatively rapid release, that is, 80% of the drug within 24 h, indicated that EGCG should be efficiently released from its vesicular formulation after localization in the tumor following intravenous administration to induce its therapeutic effect.

**Cellular uptake of EGCG**

The cellular uptake of EGCG after treatment with Tf-bearing vesicles was significantly increased (by at least 1.5-fold) in comparison with control vesicles and the drug solution for the three cell lines. It was nearly threefold higher when compared with EGCG solution on B16-F10 cells (Figures 1B & 1C). The level of drug uptake following administration of Tf-bearing vesicles was similar to that previously observed when using tocotrienol instead of EGCG as the carried drug [8].

In terms of intracellular distribution, coherent anti-Stokes Raman scattering experiments showed that EGCG was either localized in the nuclei of A431 cells or disseminated within B16-F10 and T98G cells following administration of Tf-bearing vesicles. By contrast, EGCG administered in control vesicles or as a solution could not be detected, except in T98G cells after administration of control vesicles. This might be explained by the image acquisition parameters chosen for this qualitative experiment, with a threshold probably allowing only higher amounts of EGCG to be detected.
Figure 1. Preparation and characterization of transferrin-bearing vesicles encapsulating epigallocatechin-3-gallate. (A) Transmission electron micrograph confirming the formation of vesicles encapsulating EGCG and bearing Tf. (B) Quantification of the cellular uptake of EGCG (10 µg/well) either encapsulated in Tf vesicles, control vesicles or free in solution, in A431, B16-F10 and T98G cell lines (controls: untreated cells; n = 15), respectively. Error bars represent standard error of the mean. (C) CARS and transmission microscopy imaging of the cellular uptake of EGCG (10 µg/well) either encapsulated in Tf vesicles, control vesicles or free in solution, after incubation for 24 h in A431, B16-F10 and T98G cells (controls: untreated cells). All images were taken in the size of 512 x 512 pixels and with no averaging, at a frame capture rate of 1.68 s. The pump wavelength and the Stokes beam wavelength were tuned to 752.1 and 963.4 nm, respectively, corresponding to a Raman shift of 2916 cm⁻¹ where the drug was expected to give the strongest CARS signals.

*p < 0.05.

CARS: Coherent anti-Stokes Raman scattering; EGCG: Epigallocatechin-3-gallate; Tf: Transferrin.
Antitumor activity of epigallocatechin-3-gallate encapsulated in targeted vesicles

**In vitro therapeutic efficacy**

The treatment of cells with EGCG encapsulated in Tf-bearing vesicles significantly improved the in vitro therapeutic efficacy when compared with control vesicles by 1.9-fold for A431 cells; 2.7-fold for B16-F10 cells; and fourfold for T98G cells (IC$_{50}$ ranging from 0.36 ± 0.05 to 1.41 ± 0.17 µg/ml for the Tf-bearing vesicles) (Figure 3 & Table 1). By contrast, EGCG in solution did not have significant effects on the cell viability for the experimental parameters and concentrations used; thus, demonstrating that a delivery system is necessary for the effective delivery of EGCG to cancer cells.

**In vivo tumoricidal activity**

The intravenous injection of EGCG encapsulated in Tf-bearing vesicles to the mice led to tumor regression within 24 h for both tumor types (Figures 4 & 5, Supplementary Figures 1 & 2, see online at www.futuremedicine.com/doi/suppl/10.2217/nnm.12.83). Most of these tumors continued to regress over the following month, leading to the complete disappearance of 40% of the A431 and B16-F10 tumors on day 30. Following the same treatment, 30% of A431 and 20% of B16-F10 showed a partial response, but did not entirely disappear, and 10% of B16-F10 tumors were stable. By contrast, all the A431 and B16-F10 tumors treated with control vesicles, drug solution or left untreated continued to grow.

The therapeutic effect resulting from treatment with EGCG encapsulated in Tf-bearing vesicles was also qualitatively confirmed by bioluminescence imaging on mice bearing subcutaneous B16-F10-luc tumors (Figure 6). Following treatment with the Tf-bearing formulation, luciferase expression in the tumors gradually decreased from day 1 to 7, and disappeared at day 9 as a result of the complete eradication of the tumor. By contrast, all the other treatments led to an increase of luciferase expression in the growing tumors.

This dramatically improved therapeutic effect resulted in an extended animal survival by day 26 and 25, respectively for mice bearing A431 and B16-F10 tumors, compared with untreated mice, with 60% of mice treated with Tf-bearing vesicles still being alive at day 30 (Figures 4D & 5D). The administration of control vesicles only improved animal survival by day 2 and 5, respectively for A431 and B16-F10 tumors, compared with untreated mice. This weak extension was similar to the 2-day improvement obtained following administration of EGCG solution.

No significant variation of bodyweight was detected, suggesting that all the tested EGCG formulations were well tolerated by the animals (Figures 4B & 5B).

**Discussion**

The possibility of using the green tea polyphenol, EGCG, for the treatment of cancer is hampered by the inability of this drug to reach tumors in a specific way after intravenous administration. On the basis that Tf receptors are expressed in abundance on cancer cells, and have been successfully used for the tumor targeting of therapeutic drugs and genes, the authors hypothesized that the encapsulation of EGCG in Tf-bearing vesicles would improve the delivery of this drug to cancer cells, leading to better therapeutic efficacy.

In this study, vesicles encapsulating EGCG and bearing Tf were successfully prepared. In contrast to that observed with giant unilamellar vesicles [18], the encapsulation of EGCG in the novel vesicles did not cause any shape change, aggregation or bursting of delivery systems. The vesicles used in this study were formed in a similar manner to liposomes. Their nontargeted counterparts have been used previously for the delivery of doxorubicin to tumors [19]. They were shown to alter the metabolism of this drug and to increase its therapeutic index in in vivo experiments.
The amount of EGCG encapsulated in the vesicles was measured by spectrofluorimetry. This technique has been used successfully for the detection of this drug at an excitation wavelength of 280 nm and emission wavelength of 320 nm [20]. However, the polyphenol used in this experiment was shown to present a maximum emission peak at 345 nm following excitation at 305 nm.

TF-bearing vesicles encapsulating EGCG displayed sizes enabling them to extravasate across tumor vasculature [15,16] and, therefore, have the properties required in order to efficiently deliver EGCG to tumors. The polydispersity of the vesicles led to smaller vesicles having a prolonged circulation time and higher tumor accumulation than their larger counterparts [15]; thus, highlighting the need to reduce the polydispersity and vesicle size in future experiments. EGCG has previously been encapsulated in various delivery systems, such as nanoparticles [21-24], phosphatidylethanolamine-based liposomes [25] and giant unilamellar vesicles [18]. However, to our knowledge, the present study describes, for the first time, a formulation of EGCG encapsulated in a tumor-targeted vesicular system. The resulting targeted delivery of the drug would allow a decrease of the dosage of EGCG needed to obtain a therapeutic effect.

Cellular uptake studies demonstrated that the presence of Tf at the surface of the vesicles significantly increases EGCG uptake by the three cell lines in comparison with control vesicles and EGCG solution. These results were in agreement with our previous data that showed that the use of Tf as a targeting ligand on similar vesicles improved the uptake of the drug tocotrienol by at least 1.5-fold for the three cell lines tested [8]. The nuclear and perinuclear accumulation of EGCG displayed in A431 cells treated with Tf-bearing formulation was most probably due to the cellular uptake of the drug via receptor-mediated endocytosis, as this uptake mechanism has been shown previously to give this pattern of drug distribution in the cells [26]. An incubation duration of 72 h was chosen in this study to allow comparison with the authors’ previous results obtained using similar vesicles entrapping the polyphenol tocotrienol with the same experimental settings. As the authors’ future objective is to co-entrap these two drugs in the same delivery system for a synergistic therapeutic effect, it was crucial to keep the experimental parameters of the two studies as similar as possible.

The encapsulation of EGCG in vesicles conjugated to Tf significantly improved the in vitro therapeutic efficacy of the system on the three cancer cell lines studied. These IC_{50} values were in the same range as those obtained when treating PCa and 22Tv1 prostate cancers with EGCG encapsulated in nanoparticles. In the study of Siddiqui et al., EGCG
nanoparticles (IC\textsubscript{50}; 1.71 µg/ml) presented a tenfold dose advantage compared with free EGCG (IC\textsubscript{50}; 19.9 µg/ml) on human prostate cancer cells [24]. However, the relative therapeutic effect of Tf-bearing vesicles encapsulating EGCG appears to be further improved in the three cell lines, given that the free drug was not able to kill the cancer cells at the tested concentrations and experimental parameters. These results may be attributed to the enhanced cellular uptake of EGCG when encapsulated in TF-bearing vesicles. Although this uptake was similar for the three cell lines, the in \textit{vivo} therapeutic effect was more pronounced in T98G than in the other cell lines, probably due to an increased sensitivity of T98G toward EGCG-mediated apoptosis. Das and colleagues have recently demonstrated that EGCG and other flavonoids are able to induce apoptosis in T98G cells with an increase in reactive oxygen species production and activation of kinases and proteases [27]. In A431 cells, however, EGCG-mediated apoptosis seems to involve other mechanisms, such as inhibition of NF-κB/p65, induction of DNA breaks and cleavage of poly(ADP-ribose) polymerase [28]. These various mechanisms might be responsible for the differences in cytotoxicity observed following the same treatment on these different cell lines.

In \textit{vivo}, the authors demonstrated that the intravenous administration of EGCG encapsulated within TF-bearing vesicles resulted in complete tumor eradication for 40% of B16-F10 and A431 tumors. This work corresponds to a major improvement of the therapeutic efficacy of this drug for the treatment of distant tumors. Previous \textit{in vivo} studies demonstrated that EGCG administered orally was able to inhibit the proliferation of uterine leiomyoma cells and PC-3 tumors in nude mice [29,30]. Similarly, the intraperitoneal administration of EGCG, free or encapsulated in nanoparticles, also led to a significant decrease in tumor volume compared with control mice [1,22]. Combinatorial approaches using orally administered EGCG and anticancer drugs, such as doxorubicin and erlotinib, led to a decreased tumor volume [31]. Orally administered EGCG and luteolin synergistically increased apoptosis in both Tu212 head and neck and A549 lung cancer cell lines, leading to tumor stabilization for Tu212 [32].

To the authors’ knowledge, it is the first time that the intravenous administration of EGCG encapsulated in a tumor-targeted delivery system was able to induce tumor regression, and even lead to complete tumor suppression for 40% of the treated animals on the two tested tumor types. It is also important to note that EGCG can exert an anticancer effect \textit{in vivo}, not only as a tumor growth inhibitor or as a therapeutic adjuvant, as previously described, but also by itself, provided it is efficiently targeted to the tumors.

**Conclusion**

In this study, the authors demonstrated for the first time that the green tea polyphenol, EGCG, encapsulated in a tumor-targeted delivery system can lead to complete tumor eradication for 40% of the tested tumors following intravenous administration.

The encapsulation of EGCG in TF-bearing vesicles significantly increased the cellular uptake of the drug and therefore led to an increased \textit{in vitro} therapeutic efficacy, compared with that observed with the drug in solution.

In \textit{vivo}, this tumor-targeted formulation led to complete tumor disappearance in 40% of A431 and B16-F10 tumors, tumor regression in 30% of A431 tumors and 20% of B16-F10 tumors at the end of the experiment. Animal survival was improved by more than 20 days compared with controls. By contrast, 100% of both tumor types treated with controls were growing. The treatments were well tolerated by the animals.

The green tea polyphenol, EGCG, encapsulated in TF-bearing vesicles is, therefore, a highly promising strategy for antitumor therapy and should be investigated further to optimize the anticancer therapeutic effect of this drug.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Transferrin vesicles (IC\textsubscript{50} (µg/ml) (mean ± SEM)</th>
<th>Control vesicles (IC\textsubscript{50} (µg/ml) (mean ± SEM)</th>
<th>Free EGCG (IC\textsubscript{50} (µg/ml) (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A431</td>
<td>1.41 ± 0.17</td>
<td>2.74 ± 0.39</td>
<td>&gt;25</td>
</tr>
<tr>
<td>B16-F10</td>
<td>0.96 ± 0.03</td>
<td>2.66 ± 0.57</td>
<td>&gt;25</td>
</tr>
<tr>
<td>T98G</td>
<td>0.36 ± 0.05</td>
<td>1.44 ± 1.01</td>
<td>&gt;25</td>
</tr>
</tbody>
</table>

Values are expressed as IC\textsubscript{50} values (n = 15). EGCG: Epigallocatechin-3-gallate; SEM: Standard error of the mean.
While EGCG has been widely studied for its cancer chemopreventive and therapeutic potential, little has been done regarding the tumor-specific delivery of this drug following intravenous administration. In this study, the authors demonstrated for the first time that EGCG encapsulated in a tumor-targeted
Antitumor activity of epigallocatechin-3-gallate encapsulated in targeted vesicles

A targeted delivery system was able to induce tumor regression and even tumor suppression for 40% of the treated tumors. It is hoped that these promising results will lead to the development of this field of research. The encapsulation of EGCG in tumor-targeted delivery systems should be further studied in order to reveal the full anticancer potential of this drug.

Figure 5. In vivo therapeutic efficacy of intravenously administered epigallocatechin-3-gallate formulations on B16-F10 tumors. (A) Tumor growth studies in a mouse B16-F10 tumor model after intravenous administration of Tf vesicles encapsulating EGCG (10 µg/injection); controls: control vesicles encapsulating EGCG, EGCG solution and untreated tumors (n = 10). (B) Variations of the animal bodyweight throughout the treatment. Error bars represent standard error of the mean. (C) Overall tumor response to treatments at the end of the study (day 30). (D) Time to disease progression. The y-axis gives the proportion of surviving animals over time. Animals were removed from the study once their tumor reached 12 mm in diameter.

EGCG: Epigallocatechin-3-gallate; Tf: Transferrin.
As EGCG combined with the polyphenols resveratrol and tocotrienol has been shown to elicit synergism in suppressing cell proliferation [33], the co-entrapment of these drugs in Tf-bearing vesicles to optimize the therapeutic effect is currently being worked on.

In a more general context, this study showed that a drug, previously known as a tumor growth inhibitor and as a therapeutic adjuvant, could have a therapeutic effect when targeted to tumors. It is therefore expected that some other molecules previously unable to reach the disease site may be given a second therapeutic chance as anticancer drugs following entrapment within efficacious tumor-targeted delivery systems.

Financial & competing interests disclosure
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The green tea polyphenol, epigallocatechin-3-gallate (EGCG), has promising anticancer properties. It demonstrates that the intraperitoneal administration of EGCG in transferrin-bearing vesicles improved the antiproliferative efficacy of the drug on A431, T98G and B16-F10 cells.

In vitro therapeutic efficacy
- In vitro, the encapsulation of EGCG in transferrin-bearing vesicles improved the antiproliferative efficacy of the drug on A431, T98G and B16-F10 cells.

In vivo tumoricidal activity
- In vivo, the intravenous administration of EGCG encapsulated in transferrin-bearing vesicles resulted in an entire tumor disappearance in 40% of B16-F10 and A431 tumors. Consequently, the survival of the treated mice was increased by more than 20 days compared with controls.

Conclusion
- Transferrin-bearing vesicles are highly promising for the delivery of EGCG to cancer cells in vitro and in vivo, and should be further investigated to optimize the anticancer therapeutic effect of this drug.

References
Papers of special note have been highlighted as:
* of interest
** of considerable interest

1 Tran PL, Kim SA, Choi HS, Yoon JH, Ahn SG. Epigallocatechin-3-gallate suppresses the expression of HSP70 and HSP90 and exhibits anti-tumor activity in vitro and in vivo. BMC Cancer 10, 276 (2010).


* Describes the encapsulation of EGCG in giant unilamellar vesicles.

Ethical conduct of research
The authors state that they have obtained appropriate institutional review board approval and have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.


* Describes the entrapment of EGCG in albumin nanoparticles.


* Describes the entrapment of EGCG in phosphatidylcholine-based liposomes.


* Demonstrates that combinatorial approaches using orally administered EGCG and anticancer drugs, such as doxorubicin and erlotinib, led to a decreased tumor volume.
