A receptor for green tea polyphenol EGCG

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The major polyphenol in green tea, (–)-epigallocatechin-3-gallate (EGCG), has been shown to prevent carcinogenesis. We have identified a receptor that mediates the anticancer activity of EGCG. Expression of the metastasis-associated 67-kDa laminin receptor confers EGCG responsiveness to cancer cells at physiologically relevant concentrations. Experiments using surface plasmon resonance demonstrate binding of EGCG to the 67-kDa laminin receptor with a nanomolar K_d value.

The prevention of cancer through dietary intervention is currently receiving considerable attention. Several epidemiological and animal studies suggest that green tea has a protective effect against a variety of cancer types, such as lung, prostate and breast[1]. This effect has been attributed to the biologically active polyphenol EGCG[2,3]. EGCG has both antimatrix metalloproteinase and antiangiogenesis activities[4,5] that can prevent the formation of solid tumors. The concentration of EGCG (0.1–1 μM) needed to elicit both responses is similar to levels in humans after drinking tea (usually <1 μM; ref. 6). EGCG has also been reported to inhibit cancer cell proliferation directly by affecting the signaling pathways involved in cell growth[7,8]. However, the concentrations of EGCG shown to have an effect (20–100 μM) in these previous studies are much higher than those observed in the blood or tissues. Also, the primary target for EGCG to act upon to elicit cell growth inhibition remains to be determined.

We found that all-*trans*-retinoic acid (ATRA) enhances the binding of EGCG to the cell surface of cancer cells (**Fig. 1a**) when the binding was monitored on the basis of the increase in response units in a surface plasmon resonance assay. To identify candidates through which EGCG inhibits cell growth, we used a subtraction cloning strategy involving cDNA libraries constructed from cells treated or untreated with ATRA. We isolated a single target that allows EGCG to bind to the cell surface. An analysis of the DNA sequence identified this unknown cell surface candidate as the 67-kDa laminin receptor (67 LR). In fact, the expression of this 67 LR was enhanced by ATRA treatment (**Fig. 1a**, inset). The 67 LR is expressed on a variety of tumor cells, and the expression level of this protein strongly correlates with the risk of tumor invasion and metastasis[9,10].

Human lung cancer A549 cells were used to assess how effectively the 67 LR elicits EGCG-mediated growth inhibition. Cells transfected with empty vector and treated with EGCG showed no growth inhibition (**Fig. 1b**, inset, gray bar). However, cells transfected with the

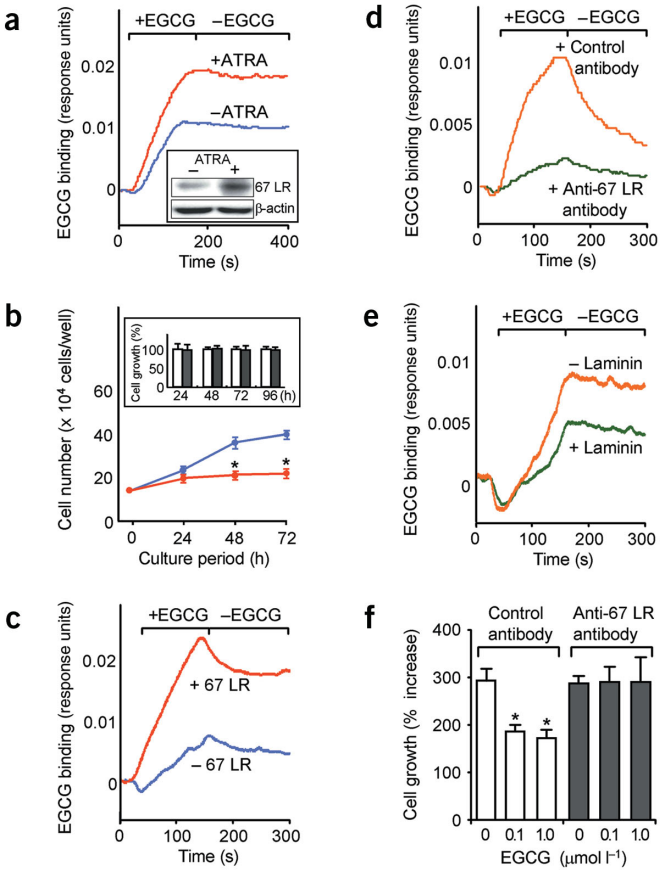

Figure 1 Anticancer action of EGCG is mediated by the 67-kDa laminin receptor. (**a**) EGCG binding to the surface of MCF-7 cells treated with (red line) or without (blue line) ATRA monitored by surface plasmon resonance. Inset, 67 LR and β-actin protein levels from the ATRA-treated or nontreated cells. (**b**) A549 cells transfected with the 67 LR vector were exposed to 5.0 μM EGCG (red line) or water (blue line) for various periods, and the cell numbers were assessed. The cells transfected with the empty vector (inset) were also treated with 5.0 μM EGCG (gray bar) or water (control, white bar), and the results are shown as relative cell number to control cultures at each period. The data presented are the mean (± s.e.m.) of triplicate experiments (asterisk indicates $P < 0.01$). (**c**) The interaction between EGCG (5.0 μM) and A549 cells transfected with the 67 LR vector (red line) or the empty vector (blue line) measured by surface plasmon resonance. (**d**) EGCG binding (1.0 μM) to the 67 LR–transfected cells treated with either anti–67 LR (green line) or control antibody (orange line). (**e**) The interaction between EGCG and 67 LR protein after treatment with either laminin (green line) or BSA (orange line). (**f**) 67 LR–transfected cells treated with either anti–67 LR (gray) or control antibody (white) were cultured in the presence of EGCG. The data presented are the mean (± s.e.m.) of triplicate experiments (asterisk indicates $P < 0.01$).

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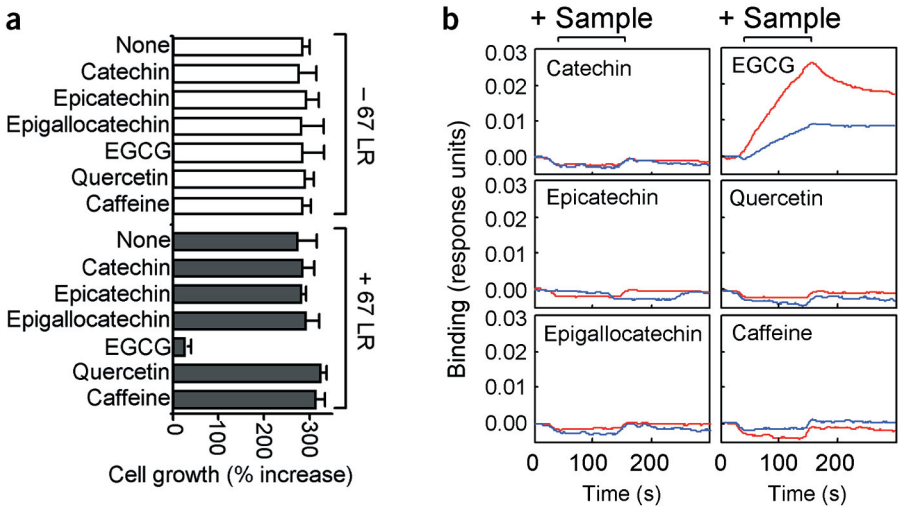

Figure 2 The interactions between tea constituents and 67 LR–transfected cells. (**a**) Growth inhibitory activities of tea constituents (indicated by bars, 5.0 μM) on cells transfected with either the gene encoding 67 LR (gray) or vector only (white) were examined and shown as indicated in **Figure 1f**. (**b**) The interaction between tea constituents (5.0 μM) and A549 cells transfected with the 67 LR vector (red line) or the empty vector (blue line) was measured using a surface plasmon resonance assay.

gene encoding 67 LR and treated with EGCG (**Fig. 1b**, red line) demonstrated considerable inhibition as compared with the cells treated with H_2O (**Fig. 1b**, blue line).

We next tested whether the growth inhibitory activity of EGCG correlates with the binding strength of EGCG to the cell surface. We found increased binding of EGCG to the cell surface of cells transfected with 67 LR (**Fig. 1c**). The number of response units did not return to the basal level after termination of EGCG exposure (**Fig. 1**, –EGCG), indicating that EGCG remains bound even in the absence of EGCG. EGCG binding to the 67 LR–transfected cells was inhibited by treatment with an antibody to 67 LR (**Fig. 1d**). We also tested whether laminin, a known ligand for the 67 LR, can compete with EGCG for binding. Laminin reduced the interaction between EGCG and the 67 LR protein (**Fig. 1e**).

We then measured the binding affinity of EGCG to 67 LR in equilibrium binding experiments using surface plasmon resonance (see **Supplementary Methods** online). K_d measurements were made with a purified recombinant 67 LR protein. The predicted K_d value for the binding of EGCG to the 67 LR protein is 39.9 nM.

To investigate whether the 67 LR can confer a sensitivity to EGCG at physiologically relevant concentrations, we treated the 67 LR–transfected cells with two concentrations of EGCG (0.1 and 1.0 μM); these concentrations are similar to the amount of EGCG found in human plasma after drinking more than two or three cups of tea[11]. The growth of the transfected cells was inhibited at both of these concentrations (**Fig. 1f**). In addition, this growth-suppressive effect was completely eliminated upon treatment with anti–67 LR before the addition of EGCG. Together, these observations demonstrate that the cell surface 67 LR is the target for EGCG and acts as the receptor for antitumor action of EGCG.

Tea also contains other biologically active compounds such as caffeine[12]. To compare the ability of 67 LR to mediate a response to other tea constituents, we examined caffeine and other tea polyphenols. None of these other compounds affected the growth of 67 LR–expressing cells (**Fig. 2a**), nor could they bind to the cell surface (**Fig. 2b**). EGCG is the only gallate (gallic acid ester) we tested, suggesting that the gallate moiety may be critical for 67 LR binding and subsequent activity.

The recent identification of a role for the 67 LR in retinal angiogenesis and of potent upregulation of this receptor in malignant mesothelioma by gene expression profiling associated with tumor endothelial cells[13] further link this receptor with the anti-angiogenic activities of EGCG. The 67 LR can bind to the prion protein and thus may regulate prion propagation[14]. The fact that EGCG can bind and regulate biological functions of the 67 LR has possible implications for prion-related diseases, for which there is currently no therapy. Ideally, increasing the expression of this target may confer a much higher EGCG potency, similar to the effect of a tumor suppressor gene. Characterizing the mechanisms by which EGCG acts through this 67 LR should help in the design of new strategies to prevent cancer.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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