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A RECEPTOR FOR EPIGALLOCATECHIN-3-*O*-GALLATE: 67 kDa LAMININ RECEPTOR MEDIATES ANTICANCER ACTION OF EGCG

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Summary
The green tea polyphenol (–)-epigallocatechin-3-gallate (EGCG) has been shown in epidemiological studies to prevent carcinogenesis, and *in vitro* studies have demonstrated its anti-proliferative and anti-angiogenic activity. The cellular target of this compound, however, has remained uncertain. Here we show that the 67 kDa laminin receptor (67LR) mediates anticancer action of EGCG[1]. We used a subtractive-cloning strategy to isolate a gene encoding a protein that allowed EGCG to bind to the cell surface. This gene was found to encode the 67LR, which is expressed on various tumor cells. Cells that were transfected with the 67LR gene grew more slowly than controls after EGCG treatment, and expression of the receptor was required for EGCG binding to cells. Furthermore, 67LR conferred sensitivity to physiological concentrations of EGCG (0.1 and 1.0 μM) which were about the same concentration found in human plasma after drinking more than two or three cups of tea. Other components of green tea, such as caffeine and other tea polyphenols, were not found to affect the growth of the 67LR-expressing cells.

Keywords
EGCG, receptor, laminin, target, cancer

Introduction
Several epidemiological studies as well as animal studies suggest that green tea has a protective effect against a variety of cancer types, such as lung, prostate and breast[2-4]. This effect has been attributed to the biologically active polyphenol, epigallocatechin-3-gallate (EGCG). EGCG has been reported to inhibit cancer cell proliferation directly by affecting the signaling pathways involved in cell growth. However, concentrations of EGCG that were shown to have an effect (20–100 μM) in these previous studies are much higher than observed in the blood or tissues. The primary target for EGCG to act upon to elicit cell growth inhibition remains to be determined.

Results & Discussion
We previously found that retinoic acid enhances EGCG effectiveness in cancer cells as well as increases the binding of EGCG to the cell surface. To identify candidates through which EGCG inhibits cell growth, we employed a subtraction cloning strategy using complementary DNA libraries constructed from cells treated with or without retinoic acid. We were able to isolate 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 67 kD laminin receptor (67 LR). 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[5]. Human lung cancer A549 cells were used to assess the effectiveness of the 67 LR for eliciting the EGCG-mediated growth inhibition. Cells transfected with 67 LR gene or vector alone and treated with H_2O showed no growth inhibition. Likewise, cells transfected with vector only and treated with EGCG also showed no growth inhibition. However, cells transfected with the 67 LR gene and treated with EGCG demonstrated remarkable inhibition (Fig. 1). We next determined whether the growth inhibitory activity of EGCG is correlated to the binding strength of EGCG to the cell surface. We found the increased

binding of EGCG to the cell surface of the cells transfected with 67LR gene (Fig. 2). We then measured the binding affinity of EGCG to 67 LR in equilibrium binding experiments using surface plasmon resonance. The predicted K_d value for the binding of EGCG to the 67 LR protein is 39.9 nM.

To investigate whether 67 LR can confer a sensitivity to EGCG at physiologically relevant concentration, we treated 67 LR-transfected cells with varying concentrations of EGCG (0.1-1.0 μM), which is similar to the amount of EGCG found in human plasma after drinking two or three cups of tea[6]. The growth of the transfected cells was inhibited at both these concentrations. Conversely, this growth suppressive effect was completely eliminated upon treatment with the 67 LR antibody 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 is also known to contain other biologically active compounds such as caffeine. To compare the ability of 67 LR to mediate a response for other tea constituents, caffeine and other tea polyphenols were examined. All these other compounds were shown to be unable to affect the growth of 67 LR-expressing cells (Fig. 3a), and also could not bind to the cell surface (Fig. 3b). Ideally, increasing the expression of this target may confer a much higher EGCG potency similar to a tumor suppressor gene. Characterizing the mechanisms by which 67LR regulates cell proliferation could provide a new approach to prevent cancer.

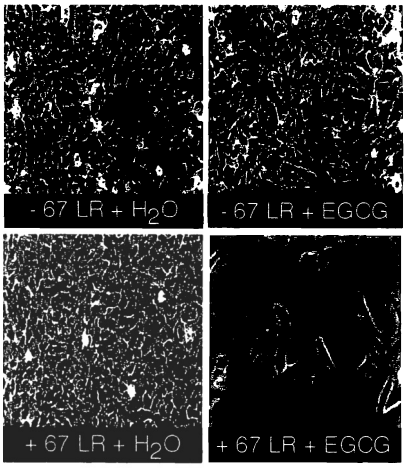

Fig. 1. Photomicrographs of A549 cell cultures transiently transfected with either vector containing the 67 LR cDNA (+ 67 LR) or the empty vector (- 67 LR) after the addition of EGCG (5 μM, + EGCG) or water (water, left).

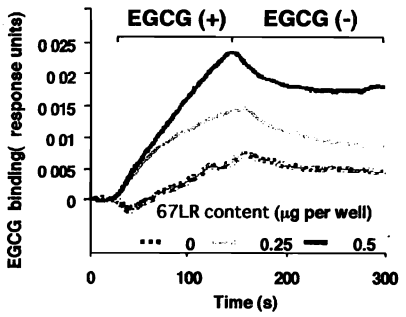

Fig. 2. The interaction between EGCG (5.0 μM) and A549 cells transfected with the 67 LR vector (black and gray lines) or the empty vector (dashed line) was measured.

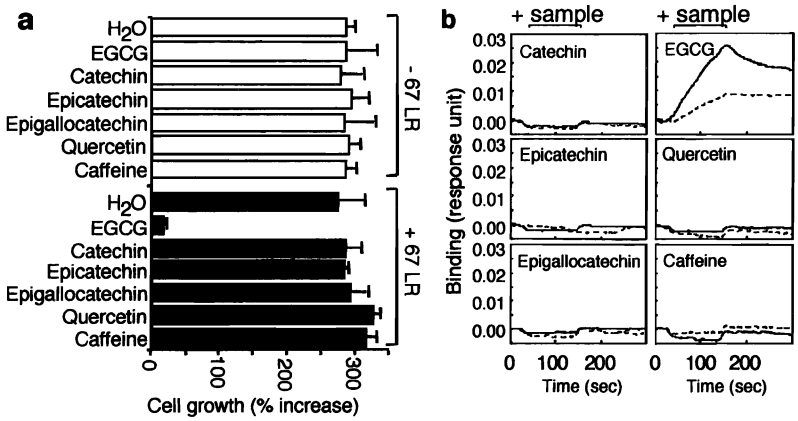

Fig. 3. The interactions between tea constituents and 67 LR-transfected cells. (**a**) Growth inhibitory activities of tea constituents (indicated each bar, 5.0 μM) on cells transfected with either the 67 LR gene (black) or vector only (white) were examined. (**b**) The interaction between tea constituents (5.0 μM) and A549 cells transfected with the 67 LR vector (line) or the empty vector (dashed line) was measured by using surface plasmon resonance assay.

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