GREEN TEA (-)-EPIGALLOCATECHIN GALLATE INHIBITS INSULIN STIMULATION OF 3T3-L1 PREADIPOCYTE MITOGENESIS VIA THE 67-KDA LAMININ RECEPTOR PATHWAY

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Objective: Insulin and (-)-epigallocatechin gallate (EGCG) have been reported to regulate fat cell mitogenesis and adipogenesis, respectively. This study investigated the pathways involved in EGCG modulation of insulin-stimulated mitogenesis in 3T3-L1 preadipocytes.

Method: 3T3-L1 preadipocytes, rat H4IIEC3 hepatoma cells, and human KB oral cancer cells were used in this study. Growth inhibition experiments. 3T3-L1 cells were plated in triplicate wells of 12-well plates. After incubation, cells were trypsinized and counted with a hemocytometer using the 0.4% trypan blue exclusion method. Cellular proliferation was performed by a commercial bromodeoxyuridine (BrdU) enzyme-linked immunosorbent assay kit. Immunoblotting and Immunoprecipitation were used for detection of the insulin receptor and its downstream signaling molecules. Cytotoxicity assay was used by measuring lactate dehydrogenase release into culture medium.

Result: EGCG inhibited insulin stimulation of preadipocyte proliferation in a dose- and time-dependent manner. EGCG also suppressed insulin-stimulated phosphorylation of the insulin receptor-β, insulin receptor (IR) substrates 1 and 2 (IRS1 and IRS2), and mitogen-activated protein kinase pathway proteins, RAF1, MEK1/2, and ERK1/2, but not JNK. Furthermore, EGCG inhibited the association of IR with the IRS1 and IRS2 proteins, but not with the IRS4 protein. These data suggest that EGCG selectively affects particular types of IRS and MAPK family members. Generally, EGCG was more effective than epicatechin, epicatechin gallate, and epigallocatechin in modulating insulin-stimulated mitogenic signaling. We identified the EGCG receptor [also known as the 67-kDa laminin receptor (67LR)] in fat cells and found that its expression was sensitive to growth phase, tissue type, and differentiation state. Pretreatment of
preadipocytes with 67LR antiserum prevented the effects of EGCG on insulin-stimulated phosphorylation of IRS2, RAF1, and ERK1/2 and insulin-stimulated preadipocyte proliferation (cell number and bromodeoxyuridine incorporation). Moreover, EGCG tended to increase insulin- stimulated associations between the 67LR and IR, IRS1, IRS2, and IRS4 proteins.

**Conclusion:** These data suggest that EGCG mediates anti-insulin signaling in preadipocyte mitogenesis via the 67LR pathway.