Arachidonic acid (AA) is a common dietary n-6 cis polyunsaturated fatty acid that under physiological conditions is present in an esterified form in cell membrane phospholipids, and it might be present in the extracellular microenvironment. AA and its metabolites are implicated in FAK activation and cell migration in MDA-MB-231 breast cancer cells, and an epithelial-to-mesenchymal-like transition process in mammary non-tumorigenic epithelial cells MCF10A. During malignant transformation is present an altered expression of glycosyltransferases, which promote changes on the glycosilation of cell-surface proteins. The β-1,4-galactosyltransferase I (GalT I) is an enzyme that participates in a variety of biological functions including cell growth, migration and spreading. However, the participation of AA in the regulation of GalT I expression and the role of this enzyme in the cell adhesion process in breast cancer cells remains to be investigated. In the present study, we demonstrate that AA induces an increase of GalT I expression through a PLA2Δ, Src, ERK1/2 and LOXs activities-dependent pathway in MDA-MB-231 breast cancer cells. Moreover, MDA-MB-231 cells adhere to laminin via GalT I expression and pretreatment of cells with AA induces and increase of cell adhesion to laminin. In conclusion, our findings demonstrate, for the first time, that AA promotes GalT I expression through an AA metabolism, Src and ERK1/2 activities-dependent pathway, and that GalT I plays a pivotal role in cell adhesion to laminin in MDA-MB-231 breast cancer cells.