Up-Regulation of E-Cadherin by an Anti-Epidermal Growth Factor Receptor Monoclonal Antibody in Lung Cancer Cell Lines

Ala-Eddin Al Moustafa,² Cedric Yansouni, Moulay A. Alaoui-Jamali, and Maureen O’Connor-McCourt

National Research Council Canada, Biotechnology Research Institute, Montréal, Québec, H4P 2R2 Canada and Department of Biochemistry, McGill University, Montréal, Québec, Canada [A-E. A., C. Y., M. O-M.]; and Lady Davis Institute for Medical Research of the Sir Mortimer B. Davis-Jewish General Hospital, Departments of Medicine and Oncology, and McGill Center for Translational Research in Cancer, Montréal, Québec, H3T 1E2 Canada [M. A. A-J.]

ABSTRACT

Many human epithelial carcinomas are characterized by the overexpression and constitutive activation of the epidermal growth factor receptor (EGF-R) via an autocrine signaling loop. We have investigated the effects of a ligand-blocking monoclonal antibody (mAb) against the EGF-R LA1 on selected parameters of human lung cancer cell lines (H322 and H661) and normal human bronchial epithelial (NHBE) cells. Using Western blot analysis, we show that H322 and NHBE cell lines express comparable levels of EGF-R/p170erbB-1. The LA1 mAb against EGF-R inhibits growth, induces differentiation to a more epithelial phenotype, reduces the constitutive activation of EGF-R, and up-regulates epithelial cadherin glycoprotein expression in H322 and NHBE cells. In contrast, LA1 had no effect on either growth, differentiation, receptor tyrosine phosphorylation, or the expression of adhesion molecules in H661 cells, which is consistent with our finding that this cell line does not express detectable levels of EGF-R. These studies demonstrate that a blocking anti-EGF-R mAb can regulate proliferation, differentiation, and the expression of cell adhesion molecules in human bronchial epithelial cells. Our findings suggest possible therapeutic avenues for the treatment of invasive carcinomas via the blockade of EGF-R with antibodies.

INTRODUCTION

Carcinomas are tumors of epithelial origin and represent over 90% of human cancers. A common feature of carcinomas is the overexpression of members of the EGF-R family, which is strongly correlated with a poor clinical prognosis (1, 2). Coexpression of EGF-R and one of its ligands, TGF-α, has also been demonstrated in human carcinomas and cell lines, suggesting a possible role for an EGF-R/TGF-α autocrine loop in human carcinogenesis (3, 4). This autocrine signaling loop and the overexpression of EGF-R have both been demonstrated in human lung cancer cells (5, 6). Anti-EGF-R-inactivating antibodies bind to EGF-R with high affinity, block the binding of EGF-R with its ligands, reduce phosphorylation of EGF-R, and inhibit the growth of epidermoid, prostate, colon, and gastric cancer cells (7–10). Accordingly, it has been suggested that inhibitors of EGF-R tyrosine kinase are potentially useful as therapeutic agents in the treatment of these cancers (11, 12).

E-cadherin is a cell-cell adhesion molecule that connects epithelial cells via homotypic calcium-dependent interactions (13). Decreased E-cadherin expression or function correlates with an enhanced aggressiveness and invasiveness of many carcinomas (14). Previous studies have revealed an inverse relationship between EGF-R activation and E-cadherin expression patterns in human oesophageal, cutaneous squamous carcinoma, and breast cancer cells (15–17).

In this study, we examined the effects of the ligand-blocking anti-EGF-R mAb LA1 on selected parameters of human lung cancer cell lines and NHBE cells. These cell lines were chosen because of their particular levels of EGF-R expression. Both the H322 and NHBE lines express comparable amounts of EGF-R, whereas none is detectable in H661 cells. We found that treatment with the LA1 antibody induces the up-regulation of E-cadherin expression, induces morphological change, inhibits cell proliferation, and reduces the constitutive activation of EGF-R in H322 and NHBE cells. This is the first demonstration that a blockade of EGF-R by an antibody results in differentiation and the up-regulation of E-cadherin. Our results suggest possible therapeutic roles for EGF-R-blocking antibodies in the treatment of invasive lung carcinomas by their ability to up-regulate the expression of cell adhesion molecules such as E-cadherin.

MATERIALS AND METHODS

Cell Culture. The human lung cancer cell lines H322 and H661 were obtained from the American Type Tissue Culture. These cell lines were cultured in DMEM with 5% FBS and incubated at 37°C in a 5% CO2 atmosphere. The NHBE cells were obtained from Clonetics (CC-2541; Normal Human Cell...
E-Cadherin and Anti-EGF-R mAb in Lung Cancer Cells

Cold PBS and lysed on ice for 30 min in lysis buffer (120 mM Tris-HCl, 135 mM NaCl, 1 mM EDTA, 1% NP40, and 0.1% SDS) supplemented with the tyrosine-phosphatase inhibitor sodium orthovanadate (1 mM) and the protease inhibitors aprotinin (10 ng/ml) and PMSF (1 mM). Nuclei and insoluble material were removed by centrifugation at 13000 g for 10 min at 4°C. Equal amounts of protein (300 μg) were precipitated with anti-EGF-R mAb (clone EGF-R; Amersham Canada Ltd.) and protein G-Sepharose (Pharmacia) overnight at 4°C. Immune complexes were separated by SDS-PAGE and immunoblotted with anti-phosphotyrosine mouse mAb 4G10 (Upstate Biotechnology, Inc.). Mouse anti-IgG1 (1 μg/ml; Becton Dickinson Canada, Inc.) was used as control for the effects of mAb LA1. Mouse anti-IgG1 (1 μg/ml) was used as control and plated over a layer of medium containing 0.7% agar. The cultures were examined every 1–2 days for 2 weeks. After multiple washings in cold PBS, 10% trichloroacetic acid, and 95% ethanol, the plates were allowed to dry for 2 hr. Cells were solubilized in 2% SDS, and the radioactivity was quantitated by liquid scintillation mixture (ICN Biomedical, Inc.)

Cell Lysis and Immunoprecipitation. Cells were grown with or without 1 μg/ml LA1 antibody for 72 h and washed with PBS. Cells were then harvested by trypsinization, washed, and fixed in 3.7% formaldehyde for 10 min. Fixed cells were hydrated in PBS, permeabilized with 0.05% Triton X-100 for 1 min, and treated with an anti-E-cadherin mAb (Uvomorulin; ICN Biomedical, Inc.) for 60 min, as previously described (18). Finally, cells were washed and resuspended in 1 ml of PBS. All steps were carried out at 4°C after trypsinization. A minimum of 1 × 10⁴ cells was analyzed using a FAC-Scan flow cytometer (Beckman Coulter).

RESULTS AND DISCUSSION

EGF-R/P170erbB-1 Expression in Human Lung Cancer and NHBE cell lines. The lung cancer cell lines H322 and H661 were chosen for comparison with NHBE cells. As shown by Western blot analysis, the H322 and NHBE cell lines express similar amounts of EGF-R (Fig. 1). EGF-R was not detectable by Western blot in H661 cells (Fig. 1). Tsao et al. (19) have previously reported that normal bronchial epithelial and immortalized HBE4-E6E7 cells express comparable levels of EGF-R mRNA. In this experiment, we show that NHBE cells and the lung cancer cell line H322 express similar levels of EGF-R protein.

The anti-EGF-R mAb LA1 Inhibits Cell Growth and Tyrosine Phosphorylation of EGF-R in H322 and NHBE Cell Lines. The present study sought to examine the effects of the anti-EGF-R LA1 neutralizing mAb on EGF-R-mediated cell activities in NHBE cells and the lung cancer cell lines H322 and H661. The H661 cell line was used as a negative control because it does not express detectable levels of EGF-R. The LA1 neutralizing mAb has been reported to compete with ligands for receptor binding (19, 20). We demonstrate that this antibody inhibits the proliferation of H322 and NHBE cell lines, whereas 1 μg/ml mouse anti-IgG1, as a control treatment, does not affect the proliferation of these cells (data not shown). Fig. 2 shows that the proliferation of both normal lung cells and H322 cancer cells relies on EGF-R activation. Conversely, the LA1 antibody does not affect the proliferation of the H661 cell line, in which there is no EGF-R, showing that the antiproliferative effect of LA1 is, indeed, mediated by blocking EGF-R (Fig. 2).
We next investigated the effect of the LA1 antibody on the activation and phosphorylation of EGF-R in H322 and NHBE cells. Immunoprecipitation with an anti-EGF-R mAb, followed by Western blotting with an antiphosphotyrosine mAb, indicated that treatment with the LA1 antibody reduces the tyrosine phosphorylation of EGF-R in the H322 and NHBE cell lines (Fig. 3). LA1 slightly up-regulates EGF-R expression in H322 and NHBE cells, but not in H661 cells (data not shown). Others have shown that anti-EGF-R-inactivating antibodies bind to EGF-R with high affinity and inhibit the growth of epidermoid, prostate, colon, and gastric cancer cells (7–10). Here we report evidence that anti-EGF-R antibodies reduce the constitutive activation of EGF-R in lung cancer and normal lung cells, resulting in inhibition of their proliferation. This study suggests that the expression and constitutive activation of the EGF-R are essential for the proliferation of both NHBE cells and cancer cells.

Effects of LA1 mAb on the Morphology and the Clonogenicity of H322, H661, and NHBE Cells. In the absence of LA1 mAb, H322, H661, and NHBE cells displayed an epithelial-like morphology. H322 and H661 form multilayered islands of cells, in contrast to NHBE cells, which form monolayers. Treatment for 3 days with 1 μg/ml LA1 caused decreases in cell proliferation and led to morphological change in the H322 and NHBE cells, but not in H661. H322 and NHBE cells flattened in appearance and presented an increase in cell-cell contacts. Fig. 4 shows the changes in H322 and H661 to a more epithelial morphology after 3 days of treatment with 1 μg/ml LA1 mAb. In contrast, EGF induces the epithelial-fibroblastoid conversion of H322 and NHBE cells, and reduces the expression of the cell-cell adhesion molecule E-cadherin (Fig. 4).4 Solc and Davies (21) have reported that EGF can induce the conversion of colon carcinoma cells to cells with a mesenchymal phenotype and reduce the adhesion molecule expression.

Fig. 2 Effect of LA1 antibody on cell growth and DNA synthesis of H322, H661, and NHBE cells. Cells (2 × 10⁴) were seeded in triplicate in 2 ml of KSF medium (NHBE) or DMEM with 5% FBS (H322 and H661), to which increasing concentrations of LA1 antibody were added (0, 0.1, 0.5, 1, and 2 μg/ml). After 3 days of treatment, DNA synthesis of all three cell types was determined by [³H]thymidine incorporation. Bars, SDs of each point.

Fig. 3 The LA1 anti-EGF-R antibody inhibits the constitutive tyrosine phosphorylation of EGF-R/P170erbB-1 (top band) in H322 and NHBE cells. No EGF-R was detected in H661 cells. Cells were grown for 3 days in either the absence (−) or presence (+) of 1 μg/ml LA1 antibody. Cell lysates were immunoprecipitated with anti-EGF-R antibody and analyzed by immunoblotting with antiphosphotyrosine antibody.

We have demonstrated that the epidermal growth factor family of ligands (EGF, TGF-α, heparin-binding EGF-like factor, and β-cellulin) induce the epithelial-fibroblastoid conversion of an immortalized human bronchial epithelial cell line (HBE4-E6E7). These ligands induce a loss of, or a reduction in, the expression of the cell-cell adhesion molecules E-cadherin and desmoplakin. This is accompanied by an overexpression of intermediate filament proteins of mesenchymal cells.5 H322 and NHBE cells were unable to form colonies in soft agar whether in the presence or absence of LA1 mAb. H661 cells displayed a very low colony-forming efficiency in agar culture. Treatment with 1 μg/ml LA1 does not affect the formation of H661 cell colonies in soft agar (data not shown).

E-cadherin Expression Is Regulated by the Anti-EGF-R mAb LA1. In epithelial tissues, E-cadherin is required for the assembly of cells into multiple layers, as well as the establishment and maintenance of an epithelial phenotype (22, 23). There is evidence that E-cadherin also acts as a suppressor of tumor invasion and metastasis (14, 24). Recent studies have reported that the activation of some receptor tyrosine kinases, including EGF-R, affects the adhesive function of E-cadherin via the β-catenin pathway (25, 26). We treated H322, H661, and NHBE cells with the LA1 antibody to determine whether the inhibition of EGF-R activation by a neutralizing mAb affects E-cadherin expression patterns. All three cell lines were treated with LA1 antibody for 3 days and examined using immunofluorescence staining with an anti-E-cadherin mAb and flow cytometric analysis. Untreated cells, as well as those treated with 1 μg/ml mouse anti-IgG1 cells, were shown to express low levels of E-cadherin. In contrast, significantly and reproducibly high lev-
els of E-cadherin were expressed in H322 and NHBE cells treated with LA1 mAb. No effect was observed in H661 cells (Fig. 5), which do not express the EGF-R. Results similar to those obtained using immunofluorescent staining were obtained using Western blot analysis with an anti-E-cadherin mAb in the same cell lines (data not shown).

The EGF-R is expressed in high levels in the majority of human lung carcinomas and cell lines (5, 6). We report here that normal NHBE and tumorigenic H322 human lung cell lines express comparable levels of EGF-R, whereas tumorigenic H661 cells express no detectable EGF-R. We examined the effects of an anti-EGF-R mAb, LA1, on these cell lines. The neutralization of EGF-R by this mAb was found to induce differentiation, inhibit proliferation, and induce the overexpression of E-cadherin only in the cell lines that express EGF-R. We observed that the LA1 antibody up-regulates E-cadherin expression in an immortalized human bronchial epithelial cell line.5 Other studies have shown that anti-EGF-R mAbs are capable of inhibiting growth of epidermoid, prostatic, colon, and gastric cancer cells (7–10). In addition, anti-EGF-R mAbs have previously been shown to induce G1 arrest and up-regulate the cyclin-dependent kinase inhibitor p27kip1 in prostatic cancer cell lines (8, 27).

Although EGF-R is overexpressed in several other types of human cancers, it is not known whether blocking EGF-R in these cell-types results in an up-regulation of E-cadherin expression similar to the effect we observe in lung cancer cells. Preliminary experiments in human breast MDA-MB-231, epidermis cancer A431, and lung cancer H460 cell lines treated with the blocking EGF-R mAb LA1, confirm our observed up-regulation of E-cadherin expression.6 A recent study of the human breast cancer cell line MDA-MB-468, by Hazan and Norton (28), shows that inactivation of EGF-R induces the interaction of E-cadherin adhesion complexes with the actin-based cytoskeleton. These authors also demonstrated a large increase in cell aggregation after monoclonal anti-EGF-R treatment, but did not show an up-regulation of E-cadherin expression.

Our study is the first evidence demonstrating that treatment with an EGF-R-inactivating mAb induces differentiation to a more epithelial phenotype and up-regulates the

Fig. 4 LA1 antibody induces a more epithelial differentiation of the H322 and NHBE, but not of the H661 cells. Untreated cells or cells treated with 1 μg/ml mouse anti-IgG1 presented an epithelial-like cell phenotype (A and B), whereas a 72-h treatment with LA1 mAb (1 μg/ml) induces a more epithelial differentiation, resulting in a flatter cell morphology (C). In contrast, a 72-h treatment with EGF (20 ng/ml) induces the epithelial-fibroblastoid conversion of H322 and NHBE cell lines, but not H661 cells (D).

6 A-E. Al Moustafa and M. O’Connor-McCourt, unpublished data.
expression of E-cadherin in normal and tumorigenic human lung cells. This observation is significant in light of the fact that a key event in carcinogenesis and metastasis is the down-regulation of cell-cell adhesion molecules, such as E-cadherin. Potential therapeutic strategies involving the up-regulation of E-cadherin by EGF-R-blocking antibodies must consider the effect we show on normal cells. Studies where control animals have been treated with anti-EGF-R mAbs have not indicated any resulting pathogenesis and suggest that these agents may not hinder normal cells (12). Finally, our results imply that the induction of E-cadherin expression by EGF-R-blocking antibodies represents a possible therapeutic strategy in the case of invasive carcinomas.

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REFERENCES


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