CANCER LETTERS

Production of epidermal growth factor related ligands in tumorigenic and benign human lung epithelial cells

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Abstract

We recently demonstrated that human lung epithelial cells, overexpressing ErbB-2, formed tumors in nude mice only when high levels of transforming growth factor α (TGFα) were produced. Cells transfected with a TGFα antisense vector failed to form tumors in nude mice. In order to further evaluate the importance, for tumorigenicity, of TGFα and its stimulation of ErbB family signalling, the production of other EGF family growth factors by these human lung epithelial cells was studied. We demonstrate for the first time that both tumorigenic and non-tumorigenic human lung epithelial cells produced, in addition to TGFα, amphiregulin, betacellulin, heparin-binding EGF and heregulin. These data suggest that human lung epithelial cells have the potential for multifactorial modulation of ErbB receptor family signalling through control of ligand as well as receptor production. In this system, the probable importance of TGFα-stimulated signaling for tumorigenicity is supported by its 13-fold higher production in tumorigenic as compared with non-tumorigenic cells and the 2-fold or lower differences observed in production of the other epidermal growth factor (EGF) family ligands. © 1999 Published by Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Cancer cells produce peptide hormones that can be secreted, bind to cell surface receptors and stimulate their own growth in an autocrine manner. The role of autocrine growth factors in the pathogenesis of lung cancer has been the subject of intensive study [1,2]. Since autocrine-stimulated mitogenesis appears to contribute significantly to disease progression, the clinical efficacy of agents that interrupt autocrine loops are currently being evaluated.

Our laboratory has been studying the role of epidermal growth factor (EGF) ligands and ErbB family receptors in the transformation of human lung epithelial cells. This growth factor family is comprised of at least six members including EGF, transforming growth factor α (TGFα), amphiregulin (AR), betacellulin (BTC), heparin-binding-EGF (HB-EGF), and several alternatively spliced isoforms of heregulin (HRG) [3]. TGFα, AR, HB-EGF and BTC compete with EGF for binding to ErbB-1 [4]. In addition, BTC also binds to ErbB-4 directly [5]. HRG binds ErbB-3 and ErbB-4 and stimulates EGFR indirectly [6]. Recent studies demonstrated that the specific patterns of downstream signaling by ErbB-1 and ErbB-2 were dependent on ligand-specified homo- or heterodimerization [7]. Thus, in understanding

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autocrine contribution to tumorigenic conversion, it is important to delineate the spectrum of ligands produced.

Production of EGF family ligands has been associated with increased proliferation in cancer. It has been demonstrated that monoclonal antibodies directed against TGFα inhibited the growth of lung adenocarcinoma cell lines in vitro [8]. Rachwal et al. have demonstrated that both HRG and TGFα mRNAs were abundantly expressed in lung tumor cell lines expressing ErbB-1 [9], and in vivo production of TGFα has been associated with shortened survival of patients with lung cancer [10]. Similarly, AR protein is expressed in 40–80% of human lung cancers and is associated with a poor prognosis for patients with non-small cell lung cancer [11]. AR mRNA has been found in lung tumor tissue, but not in adjacent benign tissue [10,12]. These studies suggest that autocrine production of EGF family ligands participates in stimulating growth of lung cancer cells.

We have recently demonstrated the importance of TGFα in inducing a tumorigenic phenotype in immortalized human lung epithelial cells. We showed that human lung epithelial cells, overexpressing ErbB-2, formed tumors in nude mice only when high levels of TGFα were produced. Inhibition of TGFα expression using an antisense strategy resulted in inhibition of tumor growth in nude mice and blocked the induction of constitutive ErbB-1/ErbB-2 heterodimers in the malignant cells [13]. We hypothesized that induction of a tumorigenic phenotype depended on achieving a threshold level of TGFα secretion. However, the role that other ErbB-1 ligands played in induction of tumorigenicity in this model was not assessed.

In the current study, we compare the production of other EGF family ligands in the tumorigenic versus non-tumorigenic cells. We demonstrate for the first time the production of a broad spectrum of EGF family ligands, HRG, AR, HB-EGF and BTC, in human lung epithelial cell lines.

2. Materials and methods

2.1. Cell line derivation and culture

The BEAS-2B cell line is a non-tumorigenic, immortalized human bronchial epithelial cell line derived from the infection of normal human bronchial epithelial cells with SV40 Adeno 12 hybrid virus [14]. It was grown in serum-free LHC-8 medium (Biofluids, Rockville, MD) according to established protocols [14]. The BEAS-2B E6 cell line was derived by introducing the human c-ErbB-2 expression vector (pLTRERBB-2neo) into BEAS-2B cells, as previously described [15]. The BEAS-2B E6T cell line (referred to as E6T) was derived from BEAS-2B E6 cells that had been passaged once in nude mice and recultured in vitro. These cells were shown to be derived from BEAS-2B by karyotypic analysis [15]. They were grown in serum-free LHC-8 medium containing geneticin (200 μg/ml) (Gibco-BRL, Gaithersburg, MD). E6TA cells were prepared by introducing a TGFα antisense expression vector (pLTRTGF-αHYG) into E6T cells, as previously described [13]. These cells were grown in serum-free LHC-8 medium containing hygromycin B (200 μg/ml) (Boehringer Mannheim, Indianapolis, IN) as well as geneticin as for E6T. MDA-MB-453 breast cancer cells (kindly provided by Ruth Lupu, Berkeley, CA) were grown in IMEM medium (Biofluids) supplemented with 10% fetal bovine serum (Biofluids). MDA-MB-231 breast cancer cells (American Type Culture Collection, Rockville, MD) were grown in RPMI-1640 medium (Biofluids) supplemented with 10% fetal bovine serum.

For basal conditions, BEAS-2B, E6T and E6TA cell lines were grown in LHC Basal medium (BioFluids) which was supplemented with insulin (5 μg/ml), transferrin (5 μg/ml) and selenium (5 ng/ml) (ITS) (Sigma, St. Louis, MO).

2.2. RNA isolation

Cells grown to 70% confluence were lysed in Trizol Reagent (Gibco-BRL, Gaithersburg, MD), and RNA isolated according to the manufacturer's protocol.

2.3. Reverse transcriptase-polymerase chain reaction

One microgram of RNA was incubated with 3 μg random hexamers, 200 units M-MLV reverse transcriptase (RT), 0.1 mM dNTP mix, 1× first strand buffer, 0.01 M dithiothreitol (DTT) (all from Gibco-BRL) and 20 units of RNasin (Promega, Madison, WI) in a total volume of 20 μl. The reaction was incubated for 40 min at 37°C, followed by 5 min at

95°C. The reverse transcribed product was used in subsequent polymerase chain reaction (PCR) protocols.

For the first amplification of HRG (isoforms α2a, β1a and β2a), primers 5′-TGAAGGAGCATAT-GTGTCTT-3′ (10 pmol) and ′-AGGAATCAGGGG-TTTCTCTGGC-3′ (10 pmol) were incubated with 10 μl of the reverse transcribed product, 1× PCR buffer (Gibco-BRL), 0.1 mM dNTP mix, 1.5 mM MgCl$_2$ (Gibco-BRL) in a total volume of 50 μl under the following conditions: one cycle of 95°C for 2 min, 35 cycles of 94°C for 1 min, 55°C for 90 s, 72°C for 2 min, followed by one cycle of 72°C for 5 min. The PCR product generated from this first round yielded a size of 805–829 bp. For the nested amplification of HRG, 10 μl of PCR product from the first PCR amplification was incubated with primers 5′-TCTGTG-TGAATGGAGGG-3′, bp 1026 (10 pmol) and 5′-GCTCTGCCGAAGACGGTCAT-3′ (10 pmol) and amplified as above under the following conditions: one cycle of 95°C for 2 min, 35 cycles of 94°C for 40 s, 50°C for 40 s, 72°C for 1 min, followed by one cycle of 72°C for 5 min. This nested PCR reaction detected the α2a isoform (277 bp), β1a isoform (292 bp) and the β2a isoform (267 bp).

For detection of the HRG β3a isoform, outer primers 5′-GTACGTCCACTCCCTTTCTGTCTCT-3′ (10 pmol) and 5′-ACAGCTTAGCAACACCCT-TTTCAG-3′ (10 pmol) were incubated with 10 μl of reverse transcribed product as above under the following conditions: one cycle of 95°C for 5 min, 35 cycles of 94°C for 40 s, 55°C for 40 s, 72°C for 90 s, followed by one cycle of 72°C for 5 min. For the nested amplification of β3a, 10 μl of PCR product from the first PCR amplification was incubated with primers 5′-GTTGGTGCTGCTTTCTTGTTGC-3′ (10 pmol) and 5′-ACAGCTTAGCAACACCCTTTT-CAG-3′ (10 pmol) and amplified as above under the following conditions: one cycle of 95°C for 5 min, 35 cycles of 94°C for 40 s, 55°C for 40 s, 72°C for 90 s, followed by one cycle of 72°C for 5 min. This nested PCR reaction yielded a product of 361 bp.

For detection of BTC and AR, primers (10 pmol) were incubated with 10 μl of reverse transcribed product, 1× PCR buffer, 0.1 mM dNTP mix, and 1.5 mM MgCl$_2$ in a total volume of 50 μl as above under the following conditions: one cycle of 95°C for 10 min, 35 cycles of 94°C for 40 s, 55°C for 30 s, 68°C

for 2 min, followed by one cycle of 72°C for 5 min. PCR reactions yielded a BTC product of 345 bp and an AR product of 478 bp. BTC primer pairs consisted of 5′-CAAGCATTACTGCATCAAAGGG-AG-3′ and 5′-CAACCTGGAGGTAACTTCATAG-CC-3′. AR primer pairs consisted of 5′-TTGGACCT-CAATGACACCTACTGTG-3′ and 5′-TGGACTTT-TCCCCACACCGTTC-3′. PCR was performed with controls lacking template, primers, and template and primers to control for contamination.

2.4. HRG bioassay

Cells were grown to 70% confluence in LHC-8 medium (Biofluids) after which the medium was replaced with LHC Basal medium (Biofluids). After 48 h, the conditioned media from each cell line were loaded onto a column containing 0.5 ml heparin-conjugated Sepharose (Sigma) equilibrated with 10 mM Tris. The column was washed once with 0.3 M NaCl in 10 mM Tris. Subsequently, 0.3 ml fractions were collected while eluting sequentially with 0.3 M NaCl, 0.6 M NaCl and 0.9 M NaCl in 10 mM Tris. The eluates were then run on NAP-5 desalting columns (Pharmacia Biotech). The resulting 1 ml fractions were concentrated to a volume of 300 μl. One hundred microlitres of each concentrated fraction was incubated for 30 min with 250 000 MDA453 cells which had been serum starved for 18 h. MDA 453 breast cancer cells express ErbB-2, ErbB-3 and ErbB-4, but not HRG [16]. The positive control for stimulation of phosphorylation was conditioned medium from MDA-MB-231 breast cancer cells which secrete four isoforms of HRG [16]. Serum-free IMEM medium (Biofluids) was used as a negative control. Following a 30 min incubation, cells were washed in serum-free media and lysed in sample buffer (Enprotech, Natick, MA). Lysates were electrophoresed on a 4–20% polyacrylamide gradient gel (Novex, San Diego, CA), transferred to Immobilon-P membranes (Millipore, Bedford, MA) and probed with anti-phosphotyrosine antibody (UBI, Lake Placid, NY) followed by anti-mouse horseradish peroxidase conjugated secondary antibody (Amersham). SuperSignal chemiluminescence reagent (Pierce, Rockford, IL) was used to develop blots according to manufacturer's protocols. Sixty microlitres of each biologically active fraction were pooled,
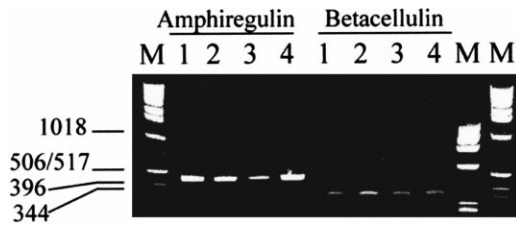
Fig. 1. RT-PCR analysis of AR and BTC. RNAs from E6T (lane 1), E6TA (lane 2), MDA-453 (lane 3) and MDA-231 (lane 4) cell lines were reverse-transcribed and amplified in a PCR reaction with primers for either AR or BTC as described. The AR PCR product is 478 bp and the BTC product 345 bp as predicted.

concentrated to a volume of 7 μl and applied to a 4–20% polyacrylamide gel for Western blot analysis. Quantitative comparisons of HRG production by different cell lines was not attempted due to the column purification where non-quantitative elution introduces uncontrolled variability between samples since partial purification eliminates any internal control marker.

2.5. Protein lysate preparation and immunoprecipitation

Cells were grown to 80% confluency in 100 mm tissue culture dishes in LHC-8 medium and then converted to basal conditions by incubation for 16 h in LHC Basal medium supplemented with insulin (5 μg/ml), transferrin (5 μg/ ml) and selenium (5 ng/ml) (ITS) (Sigma, St. Louis, MO). Cells were washed three times with cold HEPES-buffered saline (20mM HEPES in calcium-free phosphate-buffered saline with phenol red, (pH 7.5)) (Biofluids), lysed in RIPA buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% deoxycholic acid (sodium salt), 0.1% sodium dodecyl sulphate (SDS), 100 μg/ml phenyl-methylsuphonyl fluoride, 1 μg/ml aprotinin, 1 mM DTT, 1 mM sodium orthovanadate) for 10 min and scraped. The extracts were centrifuged at $40\,000 \times g$ for 30 min at 4°C. Protein concentrations were measured using the BCA method (Pierce), according to the manufacturer's instructions. For Western analysis, lysates (100μg per sample) were resolved on 8% SDS gels. For immunoprecipitation, 1 mg of lysate was incubated with 1 μg of primary antibody overnight at 4°C. Protein A/G beads (Oncogene

Science, Cambridge, MA) were added to the lysate and incubated for 1 h at 4°C. Immunoprecipitates were washed four times for 5 min each with cold PBS-0.05% Tween-20. Beads were resuspended in 2× sample buffer, heated at 95°C for 5 min and centrifuged. Supernatants were electrophoresed on 8% polyacrylamide gels. The gels were electrophoretically transferred overnight to Immobilon-P membranes. Membranes were blocked with 10% non fat dry milk in TBST (150 mM NaCl, 50 mM Tris (pH 7.5), 0.1% Tween-20) overnight followed by a 2 h incubation with primary antibody, three washes with TBST, and a 1 h incubation with horseradish peroxidase conjugated secondary antibody. Blots were developed as above. Anti-actin (Boehringer Mannheim, Indianapolis, IN), α-phosphotyrosine (UBI), α-BTC, α-AR, α-HB-EGF (R&D Systems, Minneapolis, MN), were purchased from commercial sources. Polyclonal α-HRG was a kind gift of Dr. Ruth Lupu.

2.6. Densitometry

Densitometric evaluation of Northern and Western analyses was performed on a Molecular Dynamics laser densitometer utilizing Image Quant analysis software. Multiple exposures were compared to establish linearity.

3. Results

3.1. Expression of ErbB-1 ligands and HRG

We previously demonstrated the presence of constitutive ErbB-1/ErbB-2 heterodimers in tumorigenic E6T cells [13]. Since receptor dimerization is ligand-dependent [4], and determines which downstream signaling pathways will be activated [7], we examined the presence and relative abundance of ligands for ErbB-1 in both the tumorigenic E6T and the non-tumorigenic E6TA cells. Earlier studies had shown that EGF is not produced by BEAS-2B or its ErbB-2-overexpressing derivatives [15], while TGFα secretion by E6T cells (243 pg/ml per 10^6 cells) was reduced by 93% (17 pg/ml per 10^6 cells) by expression of an antisense construct in E6TA cells [13]. We initially examined the mRNA expression of the ErbB-1 ligands AR and BTC by RT-PCR. The data of Fig. 1 reveal that mRNA for both AR and BTC was
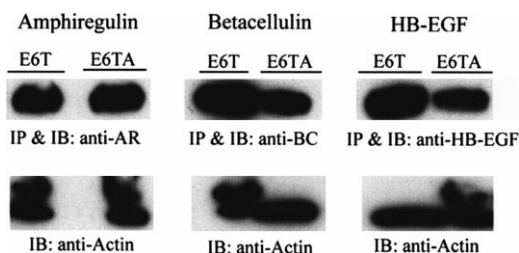
Fig. 2. Protein expression levels of AR, BTC and HB-EGF. Lysates (1 mg) of E6T and E6TA cells grown under basal conditions were immunoprecipitated with either antibodies to AR, BTC, or HB-EGF. Immunoprecipitates were analyzed by Western blotting with the indicated antibodies as described. In order to make quantitative comparisons between lysates, aliquots (50 μl) of supernatants of the first precipitation of primary antibody and protein A/G beads were analyzed by Western blotting on 8% gels with antibody to actin as described. Ratios of expression in E6T and E6TA cells are corrected for loading based on densitometric evaluation of actin in each lysate.

expressed in both E6T and E6TA cell lines. In order to relate AR levels to the expression of TGFα in E6T cells, RNA from E6T cells was evaluated for TGFα, AR and actin expression by Northern analysis and densitometric evaluation (data not shown) revealing that TGFα and AR were expressed at equivalent levels (1.0:0.93).

In order to evaluate steady state expression levels of AR, BTC and heparin-binding EGF protein (HB-EGF), lysates (1 mg) of cells grown under basal conditions were immunoprecipitated with commercially available non-cross reacting antibodies to AR, BTC or HB-EGF. Supernatants from the immunoprecipitations were immunoblotted for actin as an internal control (Fig. 2). Densitometric evaluation of actin levels in each lysate was used to correct for unequal loading. Ratios of ligand density to actin density were determined for E6T and E6TA and these values were compared. After these corrections, steady state levels of AR were indistinguishable in E6T and E6TA cells (0.94:1.0), while levels of BTC and HB-EGF were 1.6- (BTC) or 2- (HB-EGF) fold higher in E6T than in E6TA cells.

To extend the evaluation of ligands for ErbB family receptors, expression of HRG was studied by RT-PCR amplification. Only four of the possible forms of HRG generated by alternative splicing are detected in human breast cancer cells [17,18] and primers were designed to amplify these four species. HRG mRNA

was not detected by a single amplification in the E6T or E6TA cells. Therefore, one set of nested primers was designed to amplify isoforms α2a (277bp), β1a (292bp) and β2a (267bp) from RNA of E6T, E6TA and the parental BEAS-2B cell line. Only one secreted isoform (Fig. 3a) was expressed in both E6T and E6TA cell lines. The amplified species observed, most closely approximates the 277bp α2a product found in MDA-MB 231 cells (Fig. 3a). In addition, a second set of nested primers was designed to evaluate expression of the non-secreted species, β3. Primers specific for the β3 isoform amplified a product in BEAS-2B, E6T and E6TA cells (Fig. 3b). Cycle sequencing from both strands of products from the three lung cell lines confirmed the amplification of the α2a and β3 species in all cases (data not shown).

3.2. Biological activity of HRG in E6T and E6TA cells

Since detection of HRG mRNA required nested PCR amplification, the biological relevance of the expression was evaluated by testing for HRG activity in conditioned medium from BEAS-2B, E6T and E6TA cells. Previous studies have shown that HRG induces tyrosine phosphorylation of a 180-kDa protein in MDA-453 breast cancer cells which endogenously express ErbB-2, ErbB-3, and ErbB-4 in the absence of ErbB-1 and do not express HRG [16]. This assay has been used for HRG purification [16]. Therefore, MDA-453 cells were incubated with heparin–Sepharose fractions of conditioned medium of BEAS-2B, E6T and E6TA cell lines. Lysates of
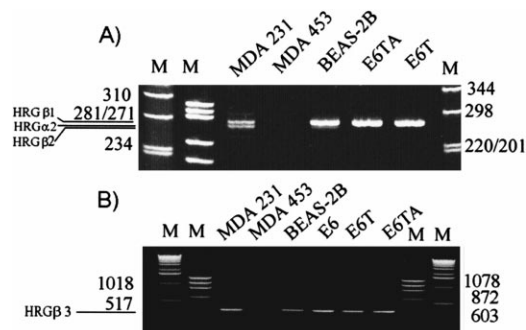

Fig. 3. RT-PCR analysis of HRG isoforms in E6T and E6TA cells. RNA (1 μg) from MDA-231, MDA-453, BEAS-2B, E6TA and E6T cells, was reverse transcribed and amplified in a PCR reaction with primers for either (a) HRG α2, β1 and β2 or (b) HRG β3 as described.
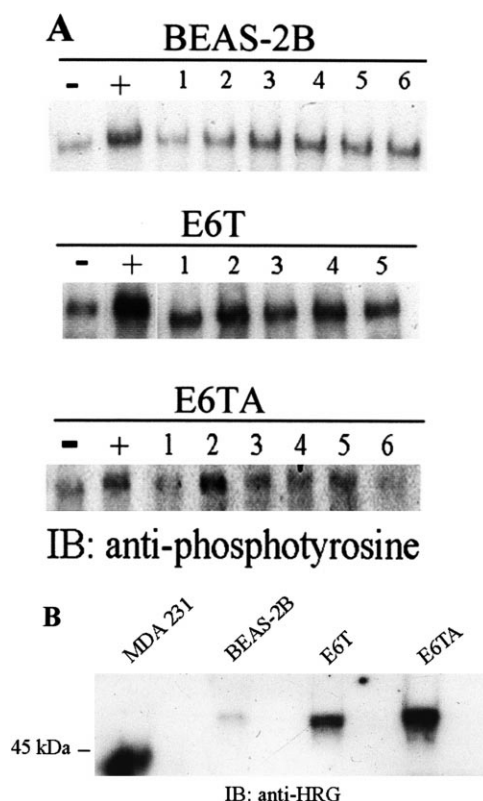
A BEAS-2B

B

IB: anti-HRG

Fig. 4. (A) HRG-like biological activity of conditioned media. Conditioned media from the indicated cell lines were tested for the ability to induce tyrosine phosphorylation of HRG receptors on the MDA-453 cell line. Conditioned media were fractionated on heparin-Sepharose columns and the ability of different fractions to stimulate the tyrosine phosphorylation of a 180-kDa protein derived from MDA-453 cells assessed. Samples 1–6 represent fractions 1–6 of the 0.9 M NaCl eluate. The negative control is serum-free IMEM and the positive control is a 1:5 dilution of unfractionated MDA-231 conditioned medium in IMEM. (B) Western analysis of HRG in conditioned media. The biologically active fractions from 4a were pooled (60 μl/fraction), concentrated to 7 μl and analyzed for the presence of HRG by immunoblotting as described.

of active fractions indicated that the total activity secreted by BEAS-2B cells was less than half that observed for E6T and E6TA. The latter two cell lines were experimentally indistinguishable. Aliquots of active fractions were pooled, concentrated and analyzed by Western analysis using a polyclonal antibody to α-HRG. As shown in Fig. 4B, the expected 45-kDa band was detected in MDA-231-conditioned medium, while the biologically active species secreted by the lung epithelial cells migrated at 55 kDa. This migration pattern is within the range observed for HRG in other cell types [16]. Comparison of HRG expression in E6T and E6TA was qualitative due to the purification steps required before evaluation of HRG protein. However, relative densities of final bands indicate that E6TA produces at least as much HRG as tumorigenic E6T and, as expected for ErbB-2-transfected cells [19], more than parental BEAS-2B cells (Fig. 4B). These data, together with the activity data, suggest that overexpression of ErbB-2 [15] may stimulate HRG secretion in these cells.

4. Discussion

Peptide growth factors of the EGF family are important modulators of the proliferation of normal and transformed human lung epithelial cells. Lung cancer cells synthesize growth factors that can potentially regulate their proliferation though autocrine pathways. Indeed, EGF family ligands control the dimerization patterns of the four ErbB receptors and, thereby, their downstream signaling patterns [7]. We have previously demonstrated that high levels of TGFα were needed to induce a tumorigenic phenotype in ErbB-1 expressing and ErbB-2 overexpressing human lung epithelial cells [13]. To examine the role of other autocrine pathways in this system, we assessed the production of other EGF-related ligands in these cell lines.

We report here that both the tumorigenic E6T and the non-tumorigenic E6TA cells express AR, BTC, HB-EGF, and HRG α2a and β3. Previous studies determined the levels of TGFα and the absence of EGF production in these cells [13,15]. Northern blotting revealed that the endogenous level of AR expression is as high as that of TGFα in E6T cells. It has been shown that AR binds to ErbB-1 with a lower

treated MDA-453 cells were prepared and analyzed by Western blotting with anti-phosphotyrosine (Fig. 4A). Active fractions of medium conditioned by MDA-231 breast cancer cells [16] served as positive controls, while serum-free medium served as a negative control. Fractions eluting at 0.9 M NaCl derived from conditioned media of E6T, E6TA, and BEAS-2B increased the phosphotyrosine content of a 180-kDa band from lysates of MDA-453 cells. While comparisons can only be qualitative, densitometric evaluation

affinity than TGFα and does not interact with ErbB 2/4 heterodimers as do other EGF family ligands [20,21]. Therefore, the biological effects of AR may not be comparable to those of TGFα, even when expression levels are similar.

Protein levels of AR, as determined by immunoblot analysis, were equivalent in E6T and E6TA cells. Similarly, Tsao et al. [22] demonstrated that parental and immortalized human bronchial epithelial cells express comparable, high levels of AR. Levels of BTC and HB-EGF were 2-fold higher in E6T as compared to E6TA cells. This difference may be insignificant in view of the 13-fold differential in TGFα production [13]. However, it is possible that higher production of these ligands may also contribute to tumorigenicity of E6T cells through increased signaling from activated ErbB family dimers.

BTC can initiate mitogenic signaling through ErbB-4 when it is expressed with ErbB-2 [5] and can also bind to ErbB-2 and ErbB-3 when these receptors are co-expressed [23,24]. While these ligand–receptor interactions would be expected to contribute to mitogenic signaling in this cell system, immuno-precipitation studies suggest that most of the ErbB-3 and ErbB-4 species present exist as heterodimers with ErbB-1 rather than ErbB-2 (Fernandes, submitted). The contribution of BTC and HB-EGF to tumorigenic conversion could be tested using an antisense approach as was used for TGFα [13].

We also examined the production of the ErbB-3/ErbB4 ligand HRG in E6T and E6TA cells. Only four (α2, β1, β2 and β3) of the possible isoforms of HRG are expressed in human breast cancer cells [17,18]. The α2a and β3 species were detected in both E6T and E6TA, but detection required nested PCR amplification suggesting a low level of expression of these ligands. Our HRG bioassay data while qualitative, do not suggest significant differences between E6T and E6TA cells in HRG levels. The apparent increase in immunologically detectable HRG in E6TA cells is puzzling. It is possible that the activity of immunologically detectable HRG derived from E6TA cells might have been masked in conditioned media by a copurifying inhibitory activity. However, since variability in elution during heparin column chromatography is expected, the experimental approach used here demonstrates the presence of HRG activity but must remain qualitative. With this caveat and the data showing similar activity levels in E6T and E6TA, we postulate that a differential expression of HRG did not contribute to the tumorigenic conversion of E6T cells.

The role that possible interactions among these growth factors play in inducing a tumorigenic phenotype has not yet been defined. It is known that different EGF ligands can synergize to enhance biological effects. For example, AR functions as an autocrine growth factor for neu-transformed human mammary epithelial cells [25]. AR has been postulated to be a mediator of EGF-induced proliferation, and AR antisense is able to inhibit the EGF induced proliferation of MCF-10A and ErbB2 transformed cells [25]. Thus, the AR expressed in our system may have potentiated the effects of TGFα in inducing a tumorigenic phenotype. However, AR is present at roughly comparable levels in tumorigenic E6T and non-tumorigenic E6TA with only HB-EGF and BTC showing a 1.6- or 2-fold differential, suggesting that any specific contribution to malignant proliferation would be dependent on TGFα.

In summary, this study reveals for the first time that both non-tumorigenic and tumorigenic human lung epithelial cells express a variety of EGF family ligands, AR, BTC, HB- EGF, and HRG α2a and β3. The activation of multiple autocrine signaling pathways thus precedes neoplastic transformation. We previously showed that TGFα production is necessary to produce a tumorigenic phenotype. The fact that human lung epithelial cells produce a broad spectrum of EGF family ligands demonstrates the importance of future studies to delineate the relative and comparative importance of these ligands in tumorigenic conversion.

Acknowledgements

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