Leptin stimulates the proliferation of human oesophageal adenocarcinoma cells via HB-EGF and TGFα mediated transactivation of the epidermal growth factor receptor

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ABSTRACT

Obesity increases the risk of developing oesophageal adenocarcinoma (OAC) as well as several other cancers. Leptin is secreted by adipocytes and serum leptin levels rise with body mass index. Leptin stimulates proliferation and inhibits apoptosis in OAC cells but the mechanisms are not fully elucidated, Transactivation of the epidermal growth factor receptor (EGFR) is an important signalling mechanism for G-protein-coupled receptors, but the relationship with leptin-type receptors has not been examined and the authors hypothesise that leptin-induced proliferation involves EGFR signalling. This study examines the effect of leptin on EGFR signalling in cultured cell lines. Leptin stimulated proliferation in four OAC lines expressing leptin receptors (OE33, OE19, BIC-1 and FLO) and this was abolished by specific EGFR inhibitors (PD153035 and AG1478). Leptin-induced proliferation was inhibited by neutralising antibodies to transforming growth factor-α (TGFα and HB-EGF) but not by anti-amphiregulin. Leptin significantly increased gene expression of HB-EGF and TGFα as measured by a quantitative real-time polymerase chain reaction (PCR) method but did not alter amphiregulin and *EGFR* gene expression. Leptin increased extracellular release of HB-EGF and TGFα and this was blocked by matrix metalloproteinase (MMP) inhibitors. The MMP inhibitors also abolished leptin-induced proliferation as well as leptin-induced EGFR tyrosine phosphorylation, but did not affect proliferation or EGFR activation induced by TGFα. The authors conclude that leptin stimulates OAC proliferation via increased gene expression of HB-EGF and TGFα, MMP-mediated extracellular release of HB-EGF and TGFα and subsequent activation of EGFR.

KEY WORDS: Amphiregulin.
Cell proliferation.
Esophageal neoplasms.
Heparin-binding EGF-like growth factor.
Leptin.
Obesity.
Receptor, epidermal growth factor.
Transforming growth factor alpha.

Introduction

The peptide hormone leptin is produced and secreted by adipose tissue, and levels are raised in proportion to body fat mass in most cases of human obesity.[1,2] Initial optimism that leptin would provide a key to unlock new treatments for human obesity unfortunately have been unfounded; although leptin undoubtedly does provide a physiological signal that contributes to the regulation of appetite and body weight (most dramatically seen in the *ob/ob* mouse, which is naturally deficient in leptin and develops gross obesity).[3] In most cases of human obesity, leptin levels are elevated and there is a degree of hypothalamic insensitivity to leptin, the mechanisms of which remain to be fully characterised.[4–6]

In contrast, an increasing body of data has shown that leptin has pathophysiological actions as a growth factor, and there is a plausible biological link between increased body mass index (BMI) and cancer. Obesity significantly increases the risk of developing several cancers, and leptin has been shown to stimulate the proliferation of a variety of obesity related cancer cell lines (breast, prostate, colon, stomach, pancreas, oesophagus).[7–15] Serum leptin levels have been shown to be an independent risk factor for the development of cancers of the colon and endometrium.[16,17] The cellular pathways responsible for transduction of these growth-promoting effects of leptin have not been fully established, but, as these are potential therapeutic targets, it is important that they are outlined.

Oesophageal adenocarcinoma (OAC) is strongly associated with obesity. The odds ratio for developing the disease in those with an obese BMI (>30 kg/m^2) is 16 compared to the leanest members of the population (BMI <22).[18,19] The authors have shown previously that leptin is a potent signal to cell proliferation and inhibition of apoptosis in oesophageal cancer cells in culture.[20] These effects are mediated via immediate activation of the extracellular signal-related kinase (p38 MAP kinase) and Akt pathways

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and the relatively late transactivation of the epidermal growth factor receptor (EGFR). Although AG1478, a widely used EGFR inhibitor, blocks the effects of leptin, there is no early increase in EGFR tyrosine phosphorylation after stimulation with leptin. In contrast, adding AG1478 after leptin inhibits OAC cell growth, and increased EGFR tyrosine phosphorylation is detectable several hours after leptin stimulation, which suggests that EGFR activation is a relatively late effect in leptin signalling.

Several other studies have demonstrated that transactivation of EGFR and subsequent downstream signalling is an essential component of pathways downstream of ligands activating their respective (non-EGFR) receptors and leading to cell proliferation, but the mechanisms underlying this have not been fully characterised.[21–23]

The present study examines the mechanisms responsible for the transactivation of EGFR by leptin in four well-characterised OAC lines. It is hypothesised that leptin increases the secretion of endogenously occurring EGFR ligands, which in turn activate EGFR. Thus, the effect of leptin is examined on the gene expression of three EGFR ligands (amphiregulin, heparin-binding epidermal growth factor [HB-EGF] and transforming growth factor–α [TGFα]) using a quantitative real-time polymerase chain reaction (RT-PCR) method, and on the extracellular release of the ligands. Well-characterised pharmacological inhibitors and neutralising antibodies directed against the EGFR ligands are used to define the pathway of leptin-induced EGFR transactivation.

Materials and methods

Cell lines and reagents

The OE33 and OE19 human oesophageal adenocarcinoma cells were obtained from ECACC (Wiltshire, UK) and the BIC-1 and FLO cells were provided by Dr. David Beer (University of Michigan, Ann Arbor, USA). Recombinant human leptin and recombinant human TGFα were obtained from Bachem (St. Helens, UK). Recombinant human amphiregulin, neutralising antibodies to human amphiregulin, human TGFα and human HB-EGF were obtained from R&D Systems (Abingdon, UK). The broad-spectrum matrix metalloproteinase (MMP) inhibitors GM6001 and MMP inhibitor-2 (N-hydroxy-1,3-di-[4-methoxybenzenesulphonyl]-5,5-dimethyl-[1,3]-piperazine-2-carboxamide), the EGFR inhibitor PD153035 and recombinant human HB-EGF were obtained from Merck (Nottingham, UK). The EGFR inhibitor AG1478 was obtained from Alexis Biochemicals (Nottingham, UK). MTT (3-[4, 5-dimethylthiazol-2-y-l]-2, 5 diphenyltetrazolium bromide) was obtained from Sigma (Poole, UK) and all other cell culture reagents were obtained from Invitrogen (Paisley, UK).

Concentrations of reagents in the final experiments were chosen based on data available in the authors' laboratory, or were chosen empirically after reviewing data from the suppliers and confirming activity.[20,23,24]

Cell culture and proliferation

All cell lines were cultured and subcultured in Dulbecco's modified Eagle's medium (DMEM) containing 4500 mg/L glucose, 100 mg/L penicillin, 100 mg/L streptomycin and 2 mmol/L L-glutamine, supplemented with 10% fetal bovine serum (FBS), as described previously.[20]

Table 1. Genes analysed by quantitative PCR.

		Sequences
Amphiregulin	Forward primer	GTGGTGCTGTCGCTCTTGATAC
	Reverse primer	GCTTCCCAGAGTAGGTTACTTG
	Probe	TCCAATCCAGCAGCATAATGGCCTGA
HB–EGF	Forward primer	TCTGGACCTTTTGAGAGTCCTTTATC
	Reverse primer	CGTGCTCCTCCTTGTTTGTT
	Probe	TCCAAGCCACAAGCACTGGCCA
TGFα	Forward primer	CTAGTTGGTTCTGGGCTTTGATCT
	Reverse primer	GGTTTTGGGCATTTGAGTCATT
	Probe	TTCCAACCTGCCCAGTCATT
EGFR	Forward primer	CGTTTGGGAGTTGATGACCTTT
	Reverse primer	GGCTGAGGGAGGCGTTCT
	Probe	AGCCATATGACGGAATCCCTGCCAG

Cell proliferation studies were performed as described previously.[20] Briefly, cells were seeded in 48-well plates and serum-stared after 24 h. Cells were then stimulated with leptin or growth factors and the modified MTT assay used as an index of relative viable cell numbers.[23] When appropriate, inhibitors or neutralising antibodies were added 60 min prior to the stimulating peptides.

Quantitative real-time polymerase chain reaction

Cells were grown in 10-cm dishes until subconfluent and then serum-starved for 24 h. Cells were then stimulated with leptin (10 ng/mL), the medium was removed at selected time-points afterwards, cells were rinsed in phosphate-buffered saline (PBS), messenger RNA (mRNA) was harvested and then reverse transcription was performed as described previously.[25] Quantitative RT-PCR was performed using the ABI Prism 770 sequence detection system (Applied Biosystems, Warrington, UK), as described previously.[25]

Primers and labelled probes (Applied Biosystems) are listed in Table 1.[25,26] All the probes contained an FAM fluorescent reporter on the 5′ end and a TAMRA quencher on the 3′ end. Control 18S ribosomal RNA (rRNA) primers and probe were obtained from Applied Biosystems. Quantitation of mRNA was performed by determining the cycle number at which amplification entered the exponential phase (termed the CT or cycle threshold) and converting these to complementary DNA (cDNA) concentrations using standard curves and comparing to the corresponding 18S rRNA levels to give arbitrary relative units, as described previously.[25]

Leptin receptor RT-PCR and Western blotting

Total mRNA was isolated and RT-PCR and detection of both the long (Ob-Rb) and short (Ob-Ra) receptor isoforms was performed, as described previously.[20] Whole-cell lysates were prepared and subjected to electrophoresis and Western blotting with anti-leptin receptor antibody (H-300, Santa Cruz Biotechnology, CA, USA, 1:200), as described previously.[20]

Quantitation of secreted EGFR ligands

OE33 cells were cultured in 24-well plates until semiconfluent. After 24-h serum-starvation they were stimulated with 10 ng/mL leptin. After 24 h, the conditioned medium was collected and secreted growth factors were measured using commercially available specific enzyme-linked immunosorbent assay (ELISA) for TGFα (Merck) and amphiregulin and HB-EGF (R&D Systems).

Epidermal growth factor receptor phosphorylation

OE33 cells were subcultured in 96-well plates and were serum-starved for 24 h once they had reached semiconfluence. Cells were then stimulated with either leptin or TGFα. EGFR phosphorylation (tyrosine-1173) was determined in 4% formaldehyde-fixed cells using a commercially available cell-based ELISA (Active Motif, Belgium), as described previously.[20]

Statistical analysis

Proliferation studies were performed in triplicate wells, EGFR phosphorylation in quadruplicate wells and EGF ligand release in duplicate wells. Results were compared to untreated control cells on the same tissue culture plate. Each experiment was performed at least three times and results are expressed as mean (±SEM). Significance was determined using Student's *t*-test, with $P<0.05$ regarded as significant.

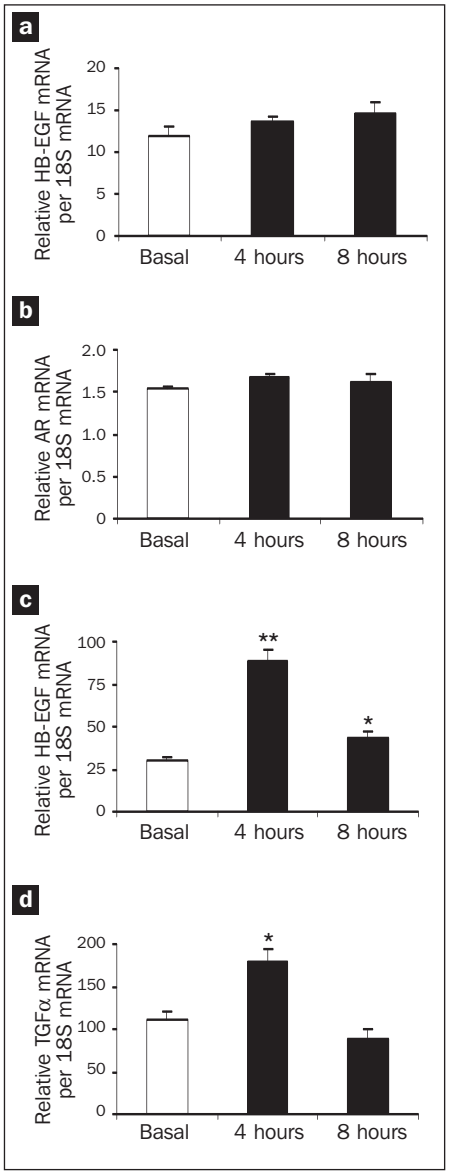

Fig. 1. Effect of leptin on EGFR and EGFR-ligand gene expression. Serum-starved OE33 cells were stimulated with 10 ng/mL leptin and gene expression of a) epidermal growth factor, b) amphiregulin, c) HB-EGF and d) TGFα were quantified by real-time PCR using specific primers and probes. Results expressed as mean (± SEM) relative to 18S mRNA levels ($n=3$, $^{*}P<0.05$ *vs*. basal, $^{**}P<0.01$ *vs*. basal).

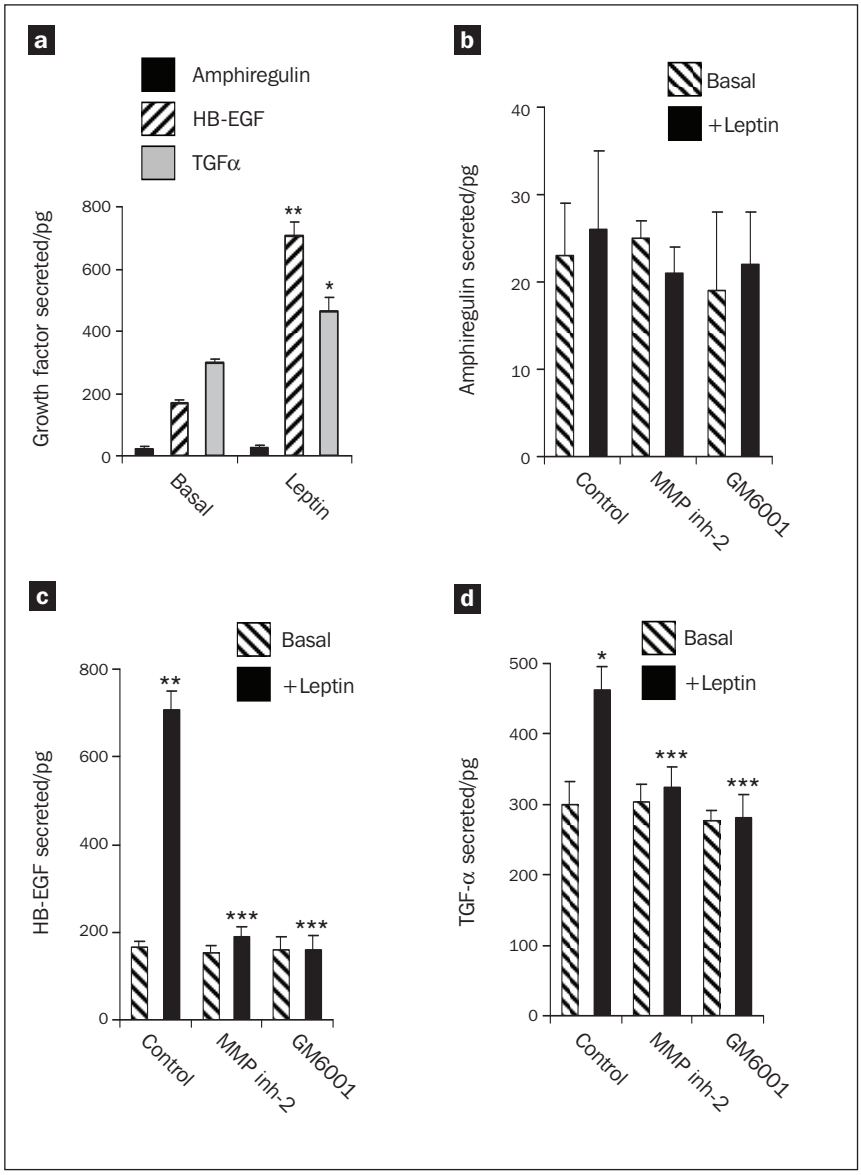

Fig. 2. Effect of leptin in EGFR-ligand secretion. Serum-starved OE33 cells were treated with 10 ng/mL leptin for 24 h. Growth factor secretion into the conditioned medium was measured using specific ELISAs. The broad-spectrum MMP inhibitors GM6001 (25 μmol/L) and MMP inh-2 (1 μmol/L) were added 60 mins prior to leptin. a) Effect of leptin on amphiregulin, HB-EGF and TGFα secretion; b) effect of MMP inhibitors on leptin-induced amphiregulin secretion; c) effect of MMP inhibitors on leptin-induced HB-EGF secretion; and d) MMP inhibitors on leptin-induced TGFα secretion. Results expressed as mean (±SEM) ($n=3$, $^{*}P<0.05$ *vs*. leptin-stimulated, $^{**}P<0.05$ *vs*. basal, $^{***}P<0.05$ *vs*. leptin-stimulated).

Results

Effect of leptin on mRNA expression of EGFR and EGFR ligands

Treatment of OE33 cells with leptin significantly increased the expression of HB-EGF and TGFα mRNA but had no effect on the mRNA levels of either amphiregulin or the EGFR (Fig. 1). HB-EGF mRNA levels increased by 183% at 4 h after leptin stimulation, and levels declined thereafter, but at 8 h after leptin stimulation the levels remained significantly elevated above control levels (35% above basal). TGFα mRNA levels also peaked at 4 h after leptin stimulation (61% above basal) but fell to control levels by 8 h after leptin stimulation. In contrast, leptin treatment did not affect the mRNA levels of either amphiregulin or EGFR.

Effect of leptin on the extracellular release of EGFR ligands

Treatment of OE33 cells with leptin significantly increased the release of both HB-EGF (by 323%) and TGFα (by 55%) in the culture medium. In contrast, leptin did not increase the release of amphiregulin (Fig. 2a). To further examine the mechanisms involved in leptin-induced EGF-ligand shedding, the effects of two different MMP inhibitors

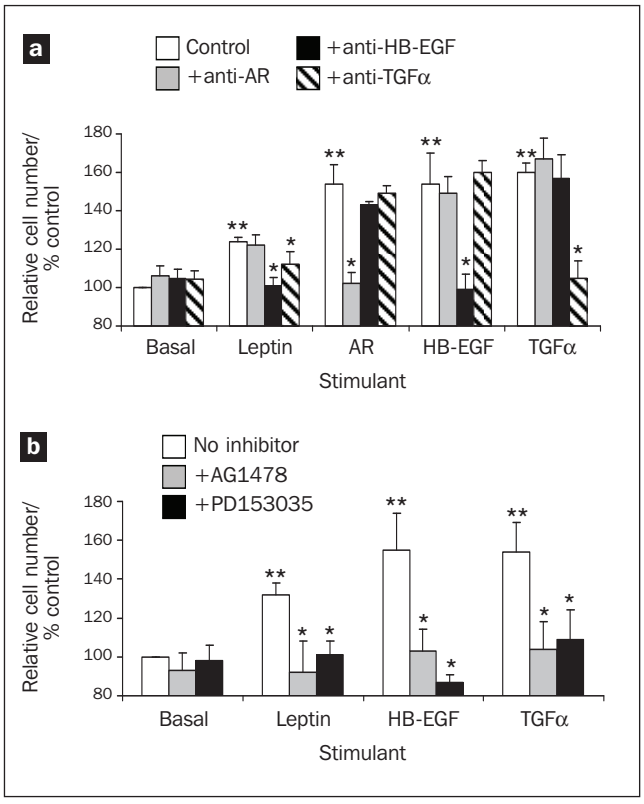

Fig. 3. Leptin-induced OE33 cell proliferation involves transactivation of the EGFR. a) Serum-starved OE33 cells were pretreated with neutralising antibodies to amphiregulin (anti-AR; 1 μg/mL), HB-EGF (10 μg/mL) or TGFα (1 μg/mL) and then stimulated with leptin (10 mg/mL) or growth factors (amphiregulin 50 ng/mL, HB-EGF 5 ng/mL, TGFα 5 ng/mL) and relative cell numbers were measured after 24 h with the MTT assay. b) Serum-starved OE33 cells were pretreated with the EGFR inhibitors AG1478 (250 nmol/L) or PD 153035 (25 nmol/L) for 60 min prior to stimulation with 10 ng/mL leptin, 5 ng/mL HB-EGF or 5 ng/mL TGFα. Relative cell numbers after 24 h were quantified using the MTT assay. Results expressed as mean (±SEM) (n=3–5, $^{*}P<0.05$ *vs*. leptin-stimulated, $^{**}P<0.05$ *vs*. basal).

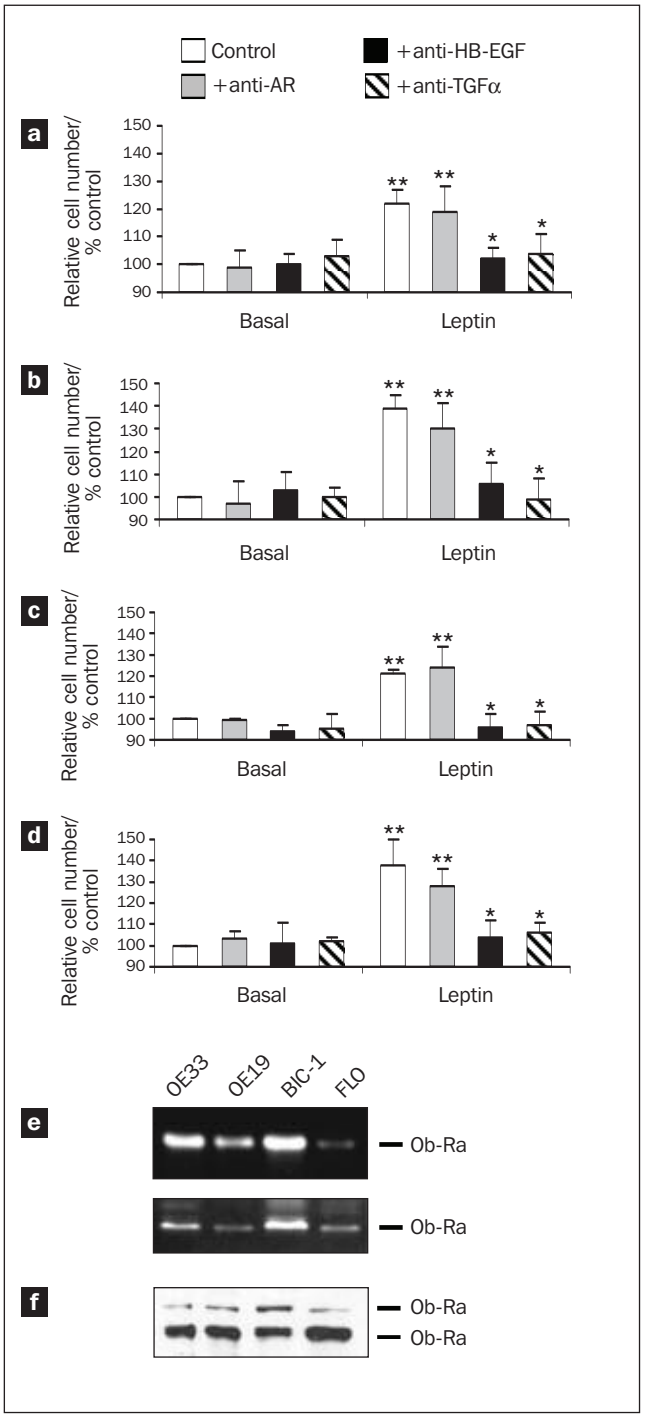

Fig. 4. Leptin-induced proliferation in oesophageal adenocarcinoma cell lines.involves HB-EGF and TGFα. Serum-starved oesophageal adenocarcinoma cells were pretreated with neutralising antibodies to amphiregulin (anti-AR 1 μg/mL, HB-EGF 10 μg/mL or TGFα 1 μg/mL) and then stimulated with leptin (10 mg/mL) and then relative cell numbers were assessed after 24 h using the MTT assay. a) Effect on OE33 cells, b) effect on OE19 cells, c) effect on BIC-1 cells, and d) effect on FLO cells. Results expressed as mean (±SEM) (n=3–5, $^{*}P<0.05$ *vs*. leptin-stimulated, $^{**}P<0.05$ *vs*. basal). e) Total mRNA from four OAC cell lines was subjected to RT-PCR for Ob-Rb and Ob-Ra using specific primers. Result is representative of three separate experiments. f) Whole-cell lysates of four oesophageal adenocarcinoma cell lines were subjected to Western blotting using an antibody directed against both the long (Ob-Rb) and short (Ob-Ra) forms of the leptin receptor. Result is representative of three separate experiments.

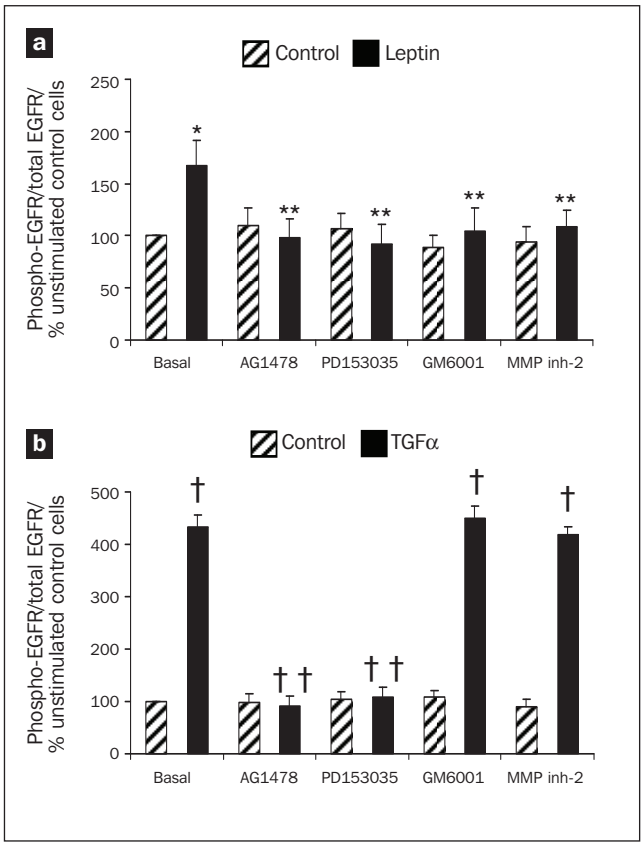

Fig. 5. MMP inhibitors prevent leptin-induced EGFR transactivation. Serum-starved OE33 cells were stimulated with a) leptin (10 ng/mL) for 6 h, or b) TGFα (5 ng/mL) for 3 min. Total and phosphorylated EGFR levels were quantified using a specific cell-based ELISA. Results are expressed as phosphorylated kinase/total kinase, normalised for cell number, compared to untreated control cells. Results expressed as mean (±SEM) (n=3, $^{*}P$<0.05 *vs*. untreated controls, $^{**}P$<0.05 *vs*. leptin-stimulated, $^{\dagger}P$<0.05 *vs*. untreated controls, $^{\dagger\dagger}P$<0.05 *vs*. TGFα-stimulated).

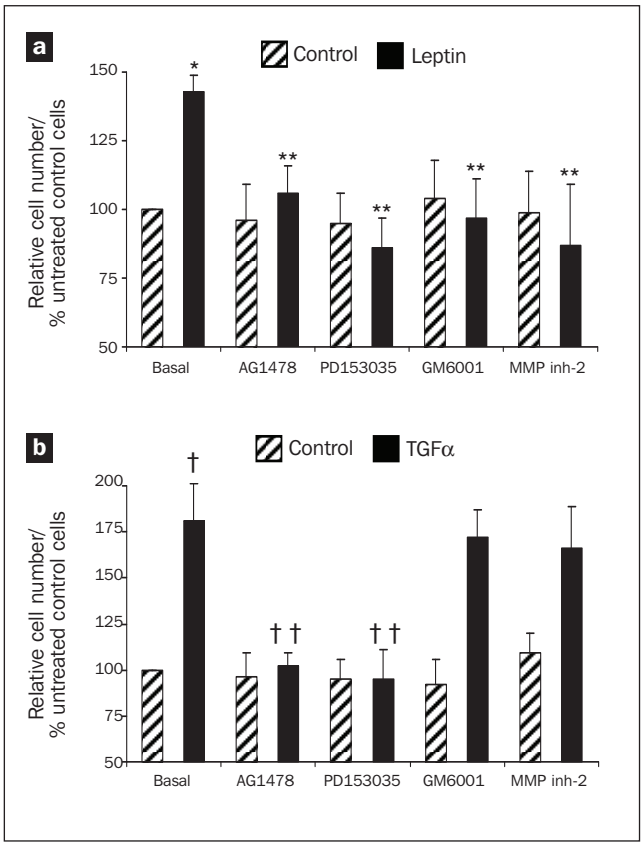

Fig. 6. MMP inhibitors prevent leptin-induced OAC cell proliferation. Serum-starved OE33 cells were pretreated with the EGFR kinase inhibitors AG1478 (250 nmol/L) or PD 153035 (25 nmol/L) or MMP inhibitors GM6001 (25 μmol/L) or MMP inh-2 (1 μmol/L) and then the stimulated with a) 10 ng/mL leptin or b) 5 ng /mL TGFα for 24 h, and then relative cell numbers were assessed by MTT assay (n=3–5, mean [±SEM], $^{*}P$<0.05 *vs*. untreated controls, $^{*}P$<0.05 *vs*. leptin-stimulated, $^{\dagger}P$<0.05 *vs*. untreated controls, $^{\dagger\dagger}P$<0.05 *vs*. TGFα-stimulated).

(GM6001 and MMP inhibitor-2) were examined. Pretreating the OE33 cells with either MMP inhbitor-2 or GM6001 abolished the extracellular shedding of both HB-EGF (Fig. 2c) and TGFα (Fig. 2d), but it had no effect on the low basal shedding of amphiregulin (Fig. 2b).

Effect of inhibition of EGFR signalling on leptin-induced proliferation

Pretreatment of OE33 cells with neutralising antibodies to HB-EGF and TGFα significantly reduced the leptin-stimulated increase in cell numbers (Fig. 3a). In contrast, pretreatment of the cells with an anti-amphiregulin neutralising antibody had no effect on leptin-stimulated cell numbers (Fig. 3a). However, recombinant human amphiregulin itself did increased OE33 cell numbers and this was blocked by the anti-amphiregulin antibody (Fig.3a). Further studies confirmed that exogenous HB-EGF and TGFα both increased OE33 cell numbers and that the neutralising antibodies to each growth factor were specific and effective at the concentrations used (Fig. 3a).

Inhibition of the kinase activity of EGFR with two separate kinase inhibitors (AG1478 and PD153035) also abolished the increase in cell numbers produced by treatment with leptin, HB-EGF or TGFα (Fig. 3b).

Effect of EGFR-ligand neutralisation in four oesophageal adenocarcinoma cell lines

To examine whether or not the pattern of leptin-induced, EGFR-ligand-mediated EGFR transactivation was similar in different OAC cell lines, duplicate experiments were performed using antibodies in four separate cell lines. The pattern of inhibition was identical. Leptin-induced proliferation in OE19 (Fig. 4b), BIC-1 (Fig. 4c) and FLO (Fig. 4d) cells to a similar degree to the OE33 cells (Fig. 4a). In all cell lines, the leptin-induced proliferation was inhibited by the anti-HB-EGF and anti-TGFα antibodies, but was unaffected by the anti-amphiregulin antibody.

Expression of the mRNA sequences was confirmed for the long (Ob-Rb) and short (Ob-Ra) leptin receptor isoforms in all four OAC cell lines (Fig. 4e). Consistent with these data, using Western blotting it was possible to detect Ob-Ra and Ob-Rb protein expression in all four cell lines (Fig. 4f). Relative expression of Ob-Ra was greater in all cell lines.

Matrix metalloproteinases are involved in leptin-induced EGFR transactivation in oesophageal adenocarcinoma cancer cells

To confirm involvement of EGFR in the growth-promoting effects of leptin, the direct phosphorylation of EGFR was

examined using a specific cell-based ELISA. It has been shown that leptin does not stimulate an increase in immediate EGFR phosphorylation and that EGFR-mediated signalling is a relatively late event in the signalling cascade.[20] In the present study, exogenous leptin increased EGFR phosphorylation by 67% at 6 h after stimulation and this was abolished by pretreatment of OE33 cells with either of the two MMP inhibitors, as well as the two different EGFR kinase inhibitors (Fig. 5a). The TGFα directly stimulated EGFR phosphorylation and, as expected, this was blocked by the two EGFR inhibitors but was unaffected by pretreatment with either GM6001 or MMP inhibitor-2 (Fig. 5b).

To confirm the sequence of signalling events related to EGFR activation leading to cell proliferation, the effects were assessed of inhibition of EGFR kinase activity and inhibition of metalloproteinases on leptin- and TGFα-stimulated cell numbers. As shown in Figure 6a, leptin-stimulated cell proliferation was completely blocked by both MMP inhibitors and both EGFR inhibitors. Direct stimulation of EGFR with TGFα significantly increased OE33 cell numbers and was unaffected by either of the MMP inhibitors, but was abolished by the two EGFR inhibitors, AG1478 and PD153035 (Fig. 6b).

Discussion

This study shows that extracellular shedding of EGF ligands and subsequent activation of EGF is an essential downstream component of leptin-induced growth promoting signalling in human oesophageal adenocarcinoma cells. Leptin had two distinct effects on EGFR ligands: induction of mRNA levels of HB-EGF and TGFα, and increased extracellular release of HB-EGF and, to a lesser extent, TGFα. Studies using the neutralising antibodies clearly showed that both HB-EGF and TGFα were essential to the cell proliferative effect of leptin. Even though the neutralising antibody abolished the growth-promoting effect of exogenous amphiregulin, it had no effect on leptin-induced proliferation, suggesting that leptin-induced EGFR transactivation does not require amphiregulin in OE33 cells. In keeping with this, leptin did not increase either amphiregulin mRNA or extracellular shedding.

Similar results with the neutralising antibodies were seen in all four OAC lines, all of which expressed leptin receptors. This suggests that this signal transduction mechanism, requiring HB-EGF and TGFα but not amphiregulin, is relatively widespread in OAC cells, although there are no data on the specific mechanisms of leptin-induced EGFR transactivation in other tissues or cell lines. Previous studies have implicated EGFR transactivation as an important signal transduction mechanism in signalling initially activated by ligands binding to other receptors, and in many of these studies the inference was indirect, utilising relatively specific EGFR inhibitors to block biological effects.[22,23]

The authors of the current study believe that their results definitely implicate EGFR in leptin-induced cell proliferation in OAC. Two distinct EGFR kinase inhibitors were used. AG1478 is probably the most widely used EGFR inhibitor and is regarded as specific, while PD153035, which appears to be an even more potent and selective agent, produced identical effects.[27] Identical inhibition of leptin-induced proliferation was obtained using specific antisera directed against the ligands that prevent ligand-induced EGFR activation via a completely different mechanism. Finally, it was confirmed that leptin itself stimulates phosphorylation of EGFR.

In is notable that leptin induced much greater increases in gene expression and extracellular shedding of HB-EGF compared to TGFα, although neutralisation of either growth factor alone was sufficient to block leptin-induced proliferation. However, exogenous administration of relatively high concentrations of HB-EGF or TGFα alone was able to enhance OE33 proliferation significantly. The explanation for this paradox is unclear but it seems unlikely to be due to lack of specificity of the antibodies used, as the stimulation experiments using exogenous growth factors showed that each of the three neutralising antibodies was specific and did not affect growth induced by the alternate EGFR ligands.

One explanation for the apparent reliance on both HB-EGF and TGFα in downstream leptin signalling is that a degree of cooperation is required between TGFα and HB-EGF signalling at the lower levels of ligand shedding induced by leptin,. The inhibitor studies showed that signalling via EGFR is essential to the downstream effects of leptin, HB-EGF and TGFα, and it is possible that although the main drive in leptin-induced signalling is through HB-EGF release, a degree of TGFα-induced signalling plays an essential permissive role, perhaps by activating a complementary pattern of EGFR autophosphorylation or by activating one of the alternate epidermal receptor forms.

Recent studies show that transactivation of EGFR contributes to the cell proliferative effects of several extracellular mediators (e.g., prostaglandins, angiotensin II, gastrin and acetylcholine) acting at muscarinic receptors. These are all typical G-protein-linked hepatohelical receptors and the mechanisms of EGFR transactivation seem to vary between tissues and cell lines, involving extracellular EGFR-ligand release as well as a more direct enzyme-mediated EGFR phosphorylation independent of extracellular ligands.[28–30]

The current study demonstrates that leptin-induced EGFR transactivation in OAC cells is dependent on extracellular shedding of HB-EGF and TGFα, and that this shedding is mediated by MMPs. Two different MMP inhibitors blocked not only leptin-induced proliferation but also leptin-induced EGFR–ligand shedding and leptin-induced EGFR phosphorylation, showing that MMP activity lies downstream of leptin activation of the leptin receptor.

Consistent with these data is the fact that neither MMP inhibitor abrogated TGFα-induced EGFR phosphorylation or TGFα-induced cell proliferation, suggesting that MMP activity is responsible for cleavage of cell surface-bound growth factors and the promotion of autocrine and paracrine EGFR transactivation. Both MMP inhibitors used have relatively broad specificity: GM6001 is known to inhibit MMP-1, -2, -3, -8 and -9, and MMP inh-2 to inhibit MMP-1, -3, -7 and -9.[24] However, the specific MMP involved in leptin-induced proliferation has yet to be determined, and further studies using more specific inhibitors and RNA interference are underway.

Similarly, it will be necessary to characterise further the mechanisms linking activation of the leptin receptor and

MMP activity and to determine whether or not there are cell-line or tissue-specific differences. Clearly, interference with this MMP/EGFR pathway in OAC has potential therapeutic benefits, either alone or as an adjunct to other therapeutic modalities such as cytotoxic chemotherapy or radiotherapy.

In conclusion, this study demonstrates a new signal transduction mechanism for leptin in OAC cells, utilising MMP-mediated extracellular shedding of HB-EGF and TGFα, resulting in ligand-mediated transactivation of EGF. In addition, leptin potently increased gene expression of HB-EGF and, to a lesser extent, TGFα. This mechanism is essential to stimulation in cell proliferation induced by leptin. This signal-transduction pathway may prove a fruitful target for therapeutic interventions in the prevention and treatment of oesophageal adenocarcinoma. □

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