Leptin and adiponectin receptor expression in oesophageal cancer

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Background: Oesophageal adenocarcinoma is an exemplar model of an obesity-associated adenocarcinoma. Altered secretion of adipokines by visceral fat is believed to play a key role in tumorigenesis. This study examined leptin receptor (ObR) and adiponectin receptor (AdipoR1 and AdipoR2) expression in oesophageal cancer, and its relationship with patient obesity status, clinicopathological data and patient survival.

Methods: Tissue microarrays were constructed from paraffin-embedded oesophagectomy specimens. ObR, AdipoR1 and AdipoR2 expression was quantified by immunohistochemistry. Anthropometric data were measured at the time of diagnosis, and obesity status was assessed using visceral fat area determined by computed tomography and body mass index. Receptor expression was correlated with various clinicopathological and anthropometric variables. Patient survival was estimated using the Kaplan–Meier method, and results compared between those with low *versus* high receptor expression. A Cox multivariable regression model was used to assess the relationship between survival and a number of co-variables.

Results: All 125 tumours analysed expressed AdipoR1 and AdipoR2, whereas 96·8 per cent expressed ObR. There was no significant difference in tumour pathological features or patient obesity status between tumours with low *versus* high ObR expression. A high level of AdipoR1 expression was significantly associated with increased patient age, obesity and less advanced tumour (T) category. Expression of AdipoR2 was inversely associated with T category ($P = 0·043$). Low AdipoR1 expression was an independent predictor of improved overall survival (hazard ratio 0·56, 95 per cent confidence interval 0·35 to 0·90; $P = 0·017$).

Conclusion: The association between adiponectin receptor expression, obesity status and tumour category and survival suggests a potential mechanism linking obesity and oesophageal cancer.

Introduction

Obesity is a strong independent predictor of incidence of and death from oesophageal adenocarcinoma[1–3]. The relative risk of developing oesophageal adenocarcinoma is 1·52 for every 5-kg/m^2 increase in body mass index (BMI)[3], and the odds ratio is 1·8 and 2·4 respectively for overweight and obese men[2]. Barrett's oesophagus, a precursor of oesophageal adenocarcinoma, has also been associated with adiposity[4,5].

The mechanisms linking obesity with oesophageal adenocarcinoma remain poorly understood. Recent studies[6–8] have shown visceral obesity to be a risk factor, and the altered secretion of adipokines such as leptin and adiponectin by visceral adipose tissue are putative key factors[9,10]. Leptin is produced mostly by adipose tissue, and circulating levels correlate positively with adipose tissue mass[11]. Leptin exerts its actions by binding to its transmembrane receptor (ObR)[10,11]. Leptin has proliferative and antiapoptotic effects in oesophageal and gastric adenocarcinoma cell lines[12,13]. Adiponectin, produced almost exclusively by adipose tissue, has insulin-sensitizing and anti-inflammatory activities, and serum adiponectin level correlates inversely with obesity status[14]. There are at least three circulating adiponectin isoforms[15] and two adiponectin receptor isoforms: AdipoR1 and AdipoR2[15]. Adiponectin is believed to play a role in the development

and progression of malignancies, including oesophageal adenocarcinoma[9,16]. Recombinant adiponectin has antiproliferative and proapoptotic effects *in vitro*[17,18]. Hypoadiponectinaemia is a risk factor for oesophageal and gastric cancer[19,20] and Barrett's oesophagus[21].

The authors[22] have demonstrated expression of ObR, AdipoR1 and AdipoR2 in oesophageal cancer at the mRNA level, and previous studies have reported the presence of adipokine receptors in gastric[23–26] and colorectal[27–30] cancer. However, there are no studies examining the protein expression of the adiponectin receptor in oesophageal cancer, and only a single small study[31] examining ObR expression. The present study had three aims: to determine whether oesophageal tumours express receptors for leptin and adiponectin at the protein level, to investigate whether receptor expression is related to obesity status, and to examine the relationship between receptor expression and patient clinicopathological characteristics and survival.

Methods

Consecutive patients undergoing oesophagectomy at St James's Hospital, Dublin, Ireland, for localized primary oesophageal cancer between 2000 and 2007 were selected. Ethical approval was obtained from the institutional review board and written informed consent obtained from all patients. Patients were excluded if there was insufficient tumour specimen to prepare the tissue microarray (TMA) or anthropometric data were insufficient. Tumour, node and metastasis (TNM) descriptors and the staging classification used were those defined in the seventh edition of the American Joint Committee on Cancer staging manual[32]. Samples were biobanked in accordance with departmental protocol[33].

Anthropometry

Anthropometric data were measured at the time of diagnosis by a single observer. Weight was measured to the nearest 0·1 kg. Height was measured to the nearest 0·5 cm. BMI was calculated as weight/height^2. Patients were asked about their bodyweight 12 months before diagnosis to allow an estimation of weight loss at diagnosis.

Radiological assessment of visceral fat area

Total, visceral (VFA) and subcutaneous fat tissue areas were calculated by a radiologist using the initial staging computed tomography (CT) image. The cross-sectional surface area of the three fat compartments at the level of the L3–L4 intervertebral disc was calculated as described previously[6,34]. Visceral obesity was defined as a VFA exceeding 80 cm^2 in women and 160 cm^2 in men[35].

Tissue samples and tissue microarray construction

TMAs were constructed from paraffin-embedded oesophagectomy specimens. The most representative areas of viable tumour were selected carefully and marked on a corresponding haematoxylin-stained slide by a pathologist; 2-mm tissue cores were then taken from these tumour-containing areas and arrayed on a paraffin block. Three cores were used for each patient.

Immunohistochemical staining

The specificity of the ObR (Santa Cruz Biotechnology, Dallas, Texas, USA), and AdipoR1 and AdipoR2 antibodies (Phoenix Pharmaceuticals, Burlingame, California, USA) was determined by western blotting. Oesophageal adenocarcinoma (OE-19 and OE-33) and squamous cell carcinoma (OE-21) cell lines were lysed and protein samples were run on 12 per cent polyacrylamide gel, and then transferred on to BioTrace™ polyvinylidene fluoride membrane (Pall Life Sciences, Ann Arbor, Michigan, USA). The membranes were probed overnight at 4°C using a 1 : 200 dilution of ObR and 1 : 500 dilutions of AdipoR1 and AdipoR2 antibodies. Single bands were obtained at 100 kDa (ObR), 50 kDa (AdipoR1) and 40 kDa (AdipoR2) (*Fig. S1*, supporting information). Immunohistochemical staining for ObR, AdipoR1 and AdipoR2 antibodies was optimized by staining 40 full-face sections of resected oesophageal cancer paraffin-embedded blocks. The sections were reviewed to ensure tissue integrity, specific staining and minimal background (non-specific) staining.

TMA slides were processed and stained manually described as previously[36]. The primary antibodies used were: for ObR, goat polyclonal antibody M-18 (Santa Cruz Biotechnology), dilution 1 : 50; for AdipoR1, rabbit polyclonal antibody 357–375 (Phoenix Pharmaceuticals), dilution 1 : 350; for AdipoR2, rabbit polyclonal antibody 374–386 (Phoenix Pharmaceuticals), dilution 1 : 350. The studies were done with avidin–biotin–peroxidase complex (Santa Cruz Biotechnology) to reveal antibody–antigen reactions. All slides were counterstained with haematoxylin. Slides were scanned digitally using ScanScope (Aperio Technologies, Vista, California, USA).

Immunohistochemical assessment

Receptor expression was graded by three independent observers, as before[37]. Grading was performed without

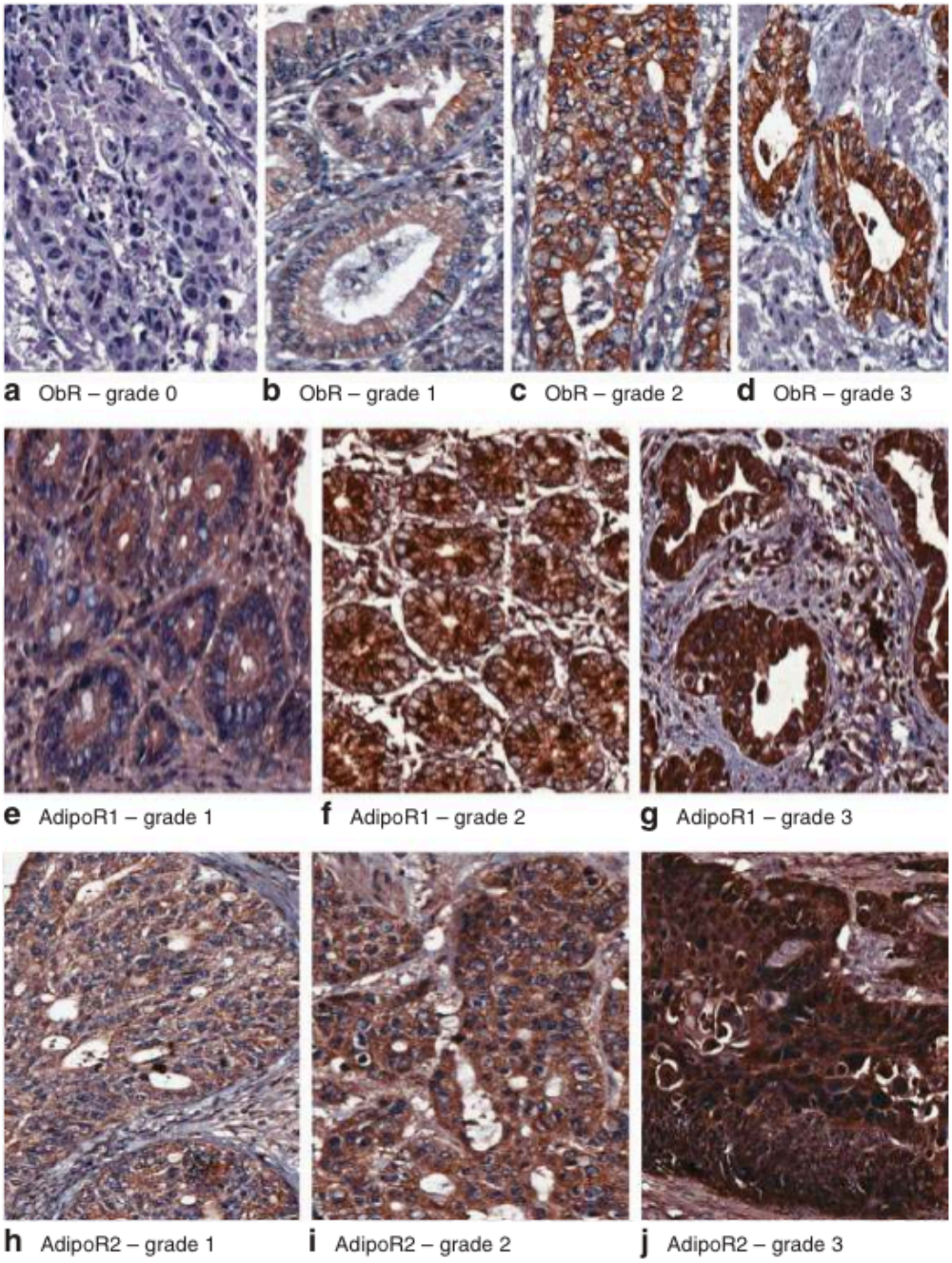

Fig. 1 Tissue microarray grading. Representative photomicrographs of immunohistochemical staining of **a–d** leptin receptor (ObR), **e–g** adiponectin receptor (AdipoR) 1 (middle row) and **h–j** AdipoR2. The intensity of receptor staining of tumour-bearing areas was scored from 0 to 3, where grade 0 was negative, grade 1 was weak, grade 2 was moderate and grade 3 represented strong staining (haematoxylin counterstain, original magnification ×20)

knowledge of patient clinical and anthropometric characteristics. Intensity of staining of tumour areas was scored from 0 to 3: grade 0, no staining (negative); grade 1, weak staining; grade 2, moderate staining; and grade 3, strong staining (*Fig. 1*). The quantity of positive staining within the core was scored between 0 and 100 per cent. The intensity score and percentage positivity were multiplied, to give an overall score for each core of between 0 and 300. The mean overall score for each of the three cores per patient was calculated.

Statistical analysis

Continuous data are expressed as mean(s.d.). Fisher's exact test (for 2×2 tables) and χ^2 test were used to evaluate statistical associations between the expression of AdipoR1, AdipoR2 and ObR in cancerous tissues, and various categorical clinicopathological and anthropometric variables, including sex, obesity status, tumour site, morphology, tumour stage, differentiation and markers of tumour invasion. One-way ANOVA was used to examine associations between receptor expression scores and continuous variables. Correlations between expression of the three receptors and BMI and VFA were assessed using the Spearman correlation coefficient.

Patient survival was estimated using the Kaplan–Meier method and a log rank test was used to compare results between patients with a low *versus* high level of receptor expression. Cut-offs for low and high expression were defined by the median expression score for each. Cox univariable and multivariable regression models were used to assess the relationship between survival and a number of co-variables, including AdipoR1, AdipoR2 and ObR expression. Results are expressed as hazard ratios with 95 per cent confidence intervals (c.i.). Statistical significance was defined as $P < 0{\cdot}050$. Statistical analyses were performed using SPSS® version 18.0 (IBM, Armonk, New York, USA).

Results

The study population consisted of 125 patients. Clinicopathological details are summarized in *Table 1*. Some 89 patients (71·2 per cent) had adenocarcinoma and 36 (28·8 per cent) squamous cell carcinoma. CT adiposity measurements were available for 61 patients. VFA measurement was not possible for 64 patients because their CT images were not available in digital format. BMI measurements were available for 103 patients; data were missing for 22 patients. Every patient had either a BMI or VFA value (or both) available to allow categorization into an obese or non-obese group. There was a strong positive correlation between BMI and CT VFA (Spearman correlation coefficient $R^2 = 0{\cdot}680$, 2-tailed $P = 0{\cdot}013$). Ninety-nine patients were aware of their baseline bodyweight. The estimated mean weight loss in the 6 months before diagnosis was 7·2(1·2) kg. The mean follow-up interval was 974(969) days.

Expression of leptin receptor in oesophageal cancer

ObR expression was observed in 121 (96·8 per cent) of the 125 tumours. Mean tumour expression of ObR was 112·5(67·8). There was a weakly positive correlation between expression of ObR and AdipoR2 (Spearman correlation coefficient $R^2 = 0{\cdot}373$, 2-tailed $P < 0{\cdot}001$). There was no significant correlation between expression of ObR and AdipoR1 ($R^2 = 0{\cdot}049$, $P = 0{\cdot}584$) or between ObR expression and patient obesity status (*Table 2*). Tumours with high ObR expression showed a trend towards being better differentiated ($P = 0{\cdot}063$). There was no significant difference in tumour pathological features, including T category, node status or markers of invasion, between tumours with a low and high ObR expression.

Table 1 Clinical, anthropometric and pathological data

	No. of patients* (n =125)
Age at diagnosis (years)†	65·7(11·0)
Sex ratio (F : M)	51 : 74
BMI (kg/m^2) (n = 103)	
Mean(s.d.)	24·5(4·1)
Underweight (BMI < 20)	18 (17·5)
Normal (BMI 20–25)	43 (41·7)
Overweight (BMI 25–30)	34 (33·0)
Obese (BMI > 30)	8 (7·8)
CT visceral fat area (cm^2) (n = 61)‡	
Mean(s.d.)	137(95)
Non-obese	34 (56)
Obese	27 (44)
Obese by any measure	
Non-obese	90 (72·0)
Obese	35 (28·0)
Tumour location	
Lower oesophagus	21 (16·8)
Middle oesophagus	18 (14·4)
Oesophageal junction	86 (68·8)
Tumour pathology	
Adenocarcinoma	89 (71·2)
Squamous cell carcinoma	36 (28·8)
Pathological tumour category	
T1	12 (9·6)
T2	31 (24·8)
T3	75 (60·0)
T4	7 (5·6)
Lymph node status	
Node-negative	42 (33·6)
Node-positive	83 (66·4)
AJCC stage	
I	15 (12·0)
II	42 (33·6)
III	68 (54·4)
Tumour differentiation	
Well	11 (8·8)
Moderate	70 (56·0)
Poor	40 (32·0)
Undifferentiated	4 (3·2)

*With percentages in parentheses unless indicated otherwise; †values are mean(s.d.). ‡Visceral obesity was defined as a visceral fat area of over 80 cm^2 in women and 160 cm^2 in men. BMI, body mass index; CT, computed tomography; AJCC, American Joint Committee on Cancer.

Table 2 Tumour leptin receptor expression and relationship with patient clinical and anthropometric variables and tumour pathology

	No. of patients	Low ObR expression ($n = 58$)	High ObR expression ($n = 67$)	P‡
Age at diagnosis (years)*	125	65·9(11·6)	65·4(10·6)	0·784§
Sex ratio (F : M)	51 : 74	25 : 33	26 : 41	0·380
BMI (kg/m^2)*	103	23·9(4·3)	25·1(3·7)	0·144§
CT visceral fat area (cm^2)*	61	128(101)	145(89)	0·481§
Visceral obesity†				0·366
Non-obese	34	19 (59)	15 (52)	
Obese	27	13 (41)	14 (48)	
Obese (any measure)				0·244
Non-obese	90	44 (76)	46 (69)	
Obese	35	14 (24)	21 (31)	
CT total fat area (cm^2)*	61	274(148)	308(138)	0·356§
CT superficial fat area (cm^2)*	61	146(86)	163(81)	0·434§
Tumour location				0·788
Lower oesophagus	21	10 (17)	11 (16)	
Middle oesophagus	18	7 (12)	11 (16)	
Oesophagogastric junction	86	41 (71)	45 (67)	
Tumour pathology				0·317
Adenocarcinoma	89	43 (74)	46 (69)	
Squamous cell carcinoma	36	15 (26)	21 (31)	
Pathological tumour category				0·096
T1 or T2	43	16 (28)	27 (40)	
T3 or T4	82	42 (72)	40 (60)	
Lymph node status				0·225
Node-negative	42	17 (29)	25 (37)	
Node-positive	83	41 (71)	42 (63)	
AJCC stage				0·242
I or II	57	24 (41)	33 (49)	
III	68	34 (59)	34 (51)	
Tumour differentiation				0·063
Low (undifferentiated/poorly differentiated)	44	25 (43)	19 (28)	
High (moderately/well differentiated)	81	33 (57)	48 (72)	
Venous invasion				0·406
No	65	29 (50)	36 (54)	
Yes	60	29 (50)	31 (46)	
Lymphatic invasion				0·480
No	38	17 (29)	21 (31)	
Yes	87	41 (71)	46 (69)	
Perineural invasion				0·176
No	86	37 (64)	49 (73)	
Yes	39	21 (36)	18 (27)	

Values in parentheses are percentages unless indicated otherwise; *values are mean(s.d.). †Visceral fat area over 80 cm^2 in women and 160 cm^2 in men. ObR, leptin receptor; BMI, body mass index; CT, computed tomography; AJCC, American Joint Committee on Cancer. ‡Fisher's exact test, except §one-way ANOVA.

Expression of adiponectin receptor 1 in oesophageal cancer

Expression of AdipoR1 was observed in all tumours. Mean tumour expression was 203·0(57·4). There was a weakly positive correlation between the expression of AdipoR1 and AdipoR2 (Spearman correlation coefficient $R^2 = 0·215$, 2-tailed $P = 0·016$). Patients with a high tumour AdipoR1 expression were older at diagnosis (67·7(11·4) *versus* 63·5(10·3) years; $P = 0·033$) (*Table 3*).

Non-obese patients were more likely to have low tumour AdipoR1 expression than the obese group ($P = 0·026$), and patients with low tumour AdipoR1 expression had lower CT VFA ($P = 0·048$) and a trend towards a lower BMI ($P = 0·066$). Low AdipoR1 expression was associated with more advanced T category ($P = 0·034$). There was no significant relationship between AdipoR1 expression and other pathological variables, such as node status, stage, degree of differentiation and markers of invasion.

Expression of adiponectin receptor 2 in oesophageal cancer

Expression of AdipoR2 was found in all tumours. Mean tumour expression of AdipoR2 was 198·1(49·5). Tumours with low AdipoR2 expression were more likely to be in

Table 3 Tumour adiponectin 1 expression and relationship with patient clinical and anthropometric variables and tumour pathology

	No. of patients	Low AdipoR1 expression ($n = 62$)	High AdipoR1 expression ($n = 63$)	P‡
Age at diagnosis (years)*	125	63·5(10·3)	67·7(11·4)	0·033§
Sex ratio (F : M)	51 : 74	24 : 38	27 : 36	0·386
BMI (kg/m^2)*	103	23·7(4·1)	25·2(3·9)	0·066§
CT visceral fat area (cm^2)*	61	111(92)	159(93)	0·048§
Visceral obesity†				0·114
Non-obese	34	19 (65·5)	15 (47)	
Obese	27	10 (34·5)	17 (53)	
Obese (any measure)				0·026
Non-obese	90	50 (81)	40 (63)	
Obese	35	12 (19)	23 (37)	
CT total fat area (cm^2)*	61	267(147)	311(138)	0·237§
CT superficial fat area (cm^2)*	61	153(83)	152(85)	0·848§
Tumour location				0·484
Lower oesophagus	21	8 (13)	13 (21)	
Middle oesophagus	18	10 (16)	8 (13)	
Oesophagogastric junction	86	44 (71)	42 (67)	
Tumour pathology				0·296
Adenocarcinoma	89	46 (74)	43 (68)	
Squamous cell carcinoma	36	16 (26)	20 (32)	
Pathological tumour category				0·034
T1 or T2	43	16 (26)	27 (43)	
T3 or T4	82	46 (74)	36 (57)	
Lymph node status				0·307
Node-negative	42	19 (31)	23 (37)	
Node-positive	83	43 (69)	40 (63)	
AJCC stage				0·160
I or II	57	25 (40)	32 (51)	
III	68	37 (60)	31 (49)	
Tumour differentiation				0·192
Low (undifferentiated/poorly differentiated)	44	19 (31)	25 (40)	
High (moderately/well differentiated)	81	43 (69)	38 (60)	
Venous invasion				0·267
No	65	30 (48)	35 (56)	
Yes	60	32 (52)	28 (44)	
Lymphatic invasion				0·181
No	38	16 (26)	22 (35)	
Yes	87	46 (74)	41 (65)	
Perineural invasion				0·328
No	86	41 (66)	45 (71)	
Yes	39	21 (34)	18 (29)	

Values in parentheses are percentages unless indicated otherwise; *values are mean(s.d.). †Visceral fat area over 80 cm^2 in women and 160 cm^2 in men. AdipoR, adiponectin receptor; BMI, body mass index; CT, computed tomography; AJCC, American Joint Committee on Cancer. ‡Fisher's exact test, except §one-way ANOVA.

a higher T category ($P = 0·043$). There were no other statistically significant relationships between AdipoR2 expression and tumour pathology, age, sex or obesity status (*Table 4*).

Tumour adipokine receptor expression and patient survival

Patients with low tumour AdipoR1 expression showed a trend towards longer overall survival ($P = 0·069$) (*Fig. S2*, supporting information). There were no significant differences in survival between patients with high and low expression of ObR or AdipoR2. Multivariable Cox regression analysis indicated that patients with low AdipoR1 expression had longer overall survival than patients with high levels of expression (hazard ratio 0·56, 95 per cent c.i. 0·35 to 0·90; $P = 0·017$) (*Table 5*). In the disease-free survival analysis, there was a trend for low AdipoR1 expression to predict prolonged disease-free survival, but this did not reach statistical significance ($P = 0·078$) (*Table 5*). There was no significant relationship between tumour ObR or AdipoR2 expression and survival (*Table 5*).

Table 4 Tumour adiponectin receptor 2 expression and relationship with patient clinical and anthropometric variables and tumour pathology

	No. of patients	Low AdipoR2 expression ($n = 67$)	High AdipoR2 expression ($n = 58$)	P‡
Age at diagnosis (years)*	125	65·1(11·3)	66·3 (10·8)	0·539§
Sex ratio (F : M)	51 : 74	30 : 37	21 : 37	0·215
BMI (kg/m^2)*	103	24·6(4·5)	24·3(3·4)	0·706§
CT visceral fat area (cm^2)*	61	138(106)	134(81)	0·886§
Visceral obesity†				0·478
Non-obese	34	19 (58)	15 (54)	
Obese	27	14 (32)	13 (46)	
Obese (any measure)				0·385
Non-obese	90	47 (70)	43 (74)	
Obese	35	20 (30)	15 (26)	
CT total fat area (cm^2)*	61	306(153)	271(131)	0·353§
CT superficial fat area (cm^2)*	61	168(92)	137(71)	0·150§
Tumour location				0·552
Lower oesophagus	21	13 (19)	8 (14)	
Middle oesophagus	18	8 (12)	10 (17)	
Oesophagogastric junction	86	46 (69)	40 (69)	
Tumour pathology				0·317
Adenocarcinoma	89	46 (69)	43 (74)	
Squamous cell carcinoma	36	21 (31)	15 (26)	
Pathological tumour category				0·043
T1 or T2	43	18 (27)	25 (43)	
T3 or T4	82	49 (73)	33 (57)	
Lymph node status				0·222
Node-negative	42	20 (30)	22 (38)	
Node-positive	83	47 (70)	36 (62)	
AJCC stage				0·136
I or II	57	27 (40)	30 (52)	
III	68	40 (60)	28 (48)	
Tumour differentiation				0·487
Low (undifferentiated/poorly differentiated)	44	23 (34)	21 (36)	
High (moderately/well differentiated)	81	44 (66)	37 (64)	
Venous invasion				0·276
No	65	37 (55)	28 (48)	
Yes	60	30 (45)	30 (52)	
Lymphatic invasion				0·520
No	38	20 (30)	18 (31)	
Yes	87	47 (70)	40 (69)	
Perineural invasion				0·176
No	86	49 (73)	37 (64)	
Yes	39	18 (27)	21 (36)	

Values in parentheses are percentages unless indicated otherwise; *values are mean(s.d.). †Visceral fat area over 80 cm^2 in women and 160 cm^2 in men. AdipoR, adiponectin receptor; BMI, body mass index; CT, computed tomography; AJCC, American Joint Committee on Cancer. ‡Fisher's exact test, except §one-way ANOVA.

Table 5 Cox regression analysis of variables predicting disease-free and overall survival in 118 patients with oesophageal cancer

	Univariable analysis		Multivariable analysis	
	Hazard ratio	P*	Hazard ratio	P*
Disease-free survival				
AdipoR1 expression (low *versus* high)	0·86 (0·56, 1·33)	0·504	0·64 (0·39, 1·05)	0·078
AdipoR2 expression (low *versus* high)	1·30 (0·84, 2·02)	0·243	1·36 (0·83, 2·24)	0·223
ObR expression (low *versus* high)	1·21 (0·78, 1·86)	0·399	0·96 (0·59, 1·55)	0·852
Overall survival				
AdipoR1 expression (low *versus* high)	0·66 (0·44, 1·03)	0·071	0·56 (0·35, 0·90)	0·017
AdipoR2 expression (low versus high)	1·15 (0·75, 1·75)	0·532	1·27 (0·78, 2·07)	0·341
ObR expression (low *versus* high)	1·16 (0·76, 1·78)	0·489	0·97 (0·60, 1·57)	0·901

Values in parentheses are 95 per cent confidence intervals. AdipoR, adiponectin receptor; ObR, leptin receptor. Co-variables include: AdipoR1, AdipoR2, ObR, age, sex, differentiation, stage of disease, and lymphatic, venous and perineural invasion. *Wald test.

Discussion

Leptin and adiponectin are putative players in the association between obesity and cancer[9,10]. In the present study, ObR, AdipoR1 and AdipoR2 protein expression was demonstrated in oesophageal tumour tissues, suggesting a potential role in oesophageal carcinogenesis. This adds to the single study reporting ObR protein expression in oesophageal cancer[31]. Oesophageal tumour expression of these receptors was demonstrated previously at the mRNA level in a series of 75 patients[22].

ObR was expressed in 96·8 per cent of tumours. Patients with high ObR expression tended to have tumours with a higher degree of pathological differentiation. This mirrors findings in oesophageal[31], gastric[38–40] and colorectal[36,41,42] cancers, where overexpression of leptin and ObR was observed in tumours with a higher degree of differentiation. It is hypothesized that the leptin system underlies a founder event in gastrointestinal tumorigenesis, and that expression may become silenced during tumour dedifferentiation. This is further supported by the finding that leptin and ObR overexpression is a positive prognostic factor and predictor of improved survival in gastric[38], colorectal[36,42,43] and hepatocellular[44] cancers. The lack of an association between ObR protein expression and obesity status or tumour stage is discordant with findings at the mRNA level[22]. These differences may relate to post-translational modifications in receptor expression. Alternatively, the discordant findings in relation to obesity status might relate to missing VFA and BMI data in some patients, and larger series may address this association.

Tumour AdipoR1 expression did, however, correlate positively with obesity status. This is consistent with findings at the mRNA level[22], suggesting that obesity-related stimuli may upregulate tumour AdipoR1 expression. There was an inverse correlation between AdipoR1 and AdipoR2 expression and T category, mirroring findings in colorectal[28], breast[45] and non-small cell lung[46] cancer. This supports the hypothesis that adiponectin inhibits tumour growth, whereby abundant receptor expression in tumour tissue facilitates the anticarcinogenic effects of adiponectin; a low level of receptor expression may permit cancer progression through loss of these protective effects.

In this study, high AdipoR1 expression independently predicted poor survival in multivariable analysis. The apparent paradox that a tumour with high adiponectin receptor expression has a worse prognosis may relate to the proangiogenic properties of adiponectin. This theory is supported by findings in a mammary tumour model in adiponectin knockout mice, in which there was slowed tumour growth, prolonged survival, reduced tumour angiogenesis and increased tumour hypoxia[47].

This study has some limitations, including the lack of availability of both BMI and CT VFA measurements in every patient, the inclusion of patients with squamous cell carcinoma, and that the relationship between receptor expression and circulating adipokine levels was not examined. This notwithstanding, the study has demonstrated that the majority of tumours express receptors for both leptin and adiponectin, that AdipoR1 is associated with obesity and is an independent predictor of patient survival, and that low expression of AdipoR1 and AdipoR2 is associated with T category. These data provide further evidence that leptin and adiponectin may be relevant to carcinogenesis and cancer biology, and that further studies exploring the link between obesity, in particular visceral fat, and key tumour pathways may be relevant to understanding of the increased prevalence of oesophageal adenocarcinoma.

Acknowledgements

This research was funded by the Cancer Clinical Trials Office, St James's Hospital, Dublin.

Disclosure: The authors declare no conflict of interest.

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