Identification of Tumor-Associated Autoantibodies in Small Cell Lung Cancer as Immune Markers of Disease

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PURPOSE

A humoral immune response in the form of autoantibodies to tumor-associated antigens had been reported in individuals with evidence of solid tumors.

These autoantibodies have been shown to be present in the circulation of individuals with lung cancer, and in some cases up to 5 years before the cancer presented clinically (1-4). These antibodies may therefore represent the earliest marker of carcinogenesis.

Whilst small cell lung cancer (SCLC) is usually diagnosed late when chances of successful treatment are poor, a potentially significant survival advantage from both early diagnosis and platinum based adjuvant chemotherapy has been reported (5). A test that could identify such cancers at an early stage is critical to increase the chance of successful treatment.

To our knowledge this is the first large study specifically looking at individuals with small cell lung cancer (SCLC) compared with age and smoking matched control sera.

METHODS

Serum from patients with SCLC (n=242), 5 individuals deemed to be at a high risk of developing a SCLC, as well as age, sex and smoking matched normal control sera, were investigated for the presence of autoantibodies to a panel of tumor-associated antigens.

Patient Details and Clinical Characteristics

	ALL SCLC	SCLC-ED	SCLC-LD	At Risk	Normal
Group - n	243	153	90	4	247
Mean age (range)	66 (33-87)	66 (43-86)	66 (33-87)	69 (55-87)	66 (33-87)
Female % (n)	49% (119)	46% (71)	53% (48)	50% (2)	49% (121)
Smoker/Ex %	99%	99%	99%	100%	99%

ED: Extensive Disease; LD: Limited Disease (lymph node –ve: n=20)
LD stages: Stage 1 (n=14, 1A : n=5), Stage 2 (n=15), Stage 3 (n=61)

ASSAY PROCEDURE

Serum samples were evaluated for autoantibodies to a panel of six recombinant cancer-associated antigens (p53, NY-ESO-1, CAGE, GBU4-5, SOX2 and HuD) using a semi-automated enzyme-linked immunosorbent assay (ELISA) method where optical densities (OD) were converted to calibrated reference units (RU) (1). Full assay details are described elsewhere (1).

RESULTS

Elevated levels of autoantibodies were seen to at least 1 of 6 antigens in 43% of all the SCLC patient sera tested, with a specificity of 99%; or 55% at a specificity of 90%. These was no significant difference in sensitivity by stage of the disease or nodal involvement.

Individual autoantibodies were raised in up to 35% of all SCLC samples at an individual antigen specificity of at least 97%.

Frequency of Autoantibodies to Tumor-Associated Antigens

Sensitivity for Panel Specificity of 99%

Group		SOX2 %	HuD %	p53 %	NY-ESO-1 %	CAGE %	GBU 4-5 %	PANEL %
All SCLC	+ve 95% CI	29*** (23, 35)	9*** (6, 14)	12*** (8, 16)	3* (1, 6)	4* (2, 7)	1^{NS} (0, 3)	42*** (36, 48)
Limited Disease	+ve 95% CI	29*** (20, 39)	10*** (5, 18)	13*** (7, 22)	3^{NS} (1, 9)	0 (0, 4)	1^{NS} (0, 6)	41*** (31, 52)
Extensive Disease	+ve 95% CI	29*** (22, 37)	9*** (5, 15)	10*** (6, 16)	3* (1, 7)	6* (3, 11)	1^{NS} (0, 4)	42*** (35, 51)
Matched Normals	+ve 95% CI	0 (0, 1)	0 (0, 1)	<1 (0, 2)	1 (0, 3)	0 (0, 1)	0 (0, 1)	1 (0, 3)
Specificity %		**100**	**100**	**>99**	**99**	**100**	**100**	**99**

Sensitivity for Panel Specificity of 90%

Group		SOX2 %	HuD %	p53 %	NY-ESO-1 %	CAGE %	GBU 4-5 %	PANEL %
All SCLC	+ve 95% CI	35*** (29, 41)	13*** (9, 18)	16*** (12, 21)	6^{NS} (3, 10)	7* (4, 11)	4* (2, 7)	55*** (48, 61)
Limited Disease	+ve 95% CI	36*** (26, 46)	17*** (10,26)	17*** (10, 26)	4^{NS} (1, 11)	3^{NS} (1, 9)	3^{NS} (1, 9)	53*** (43, 64)
Extensive Disease	+ve 95% CI	34*** (27, 42)	11*** (7, 17)	16*** (10, 22)	7^{NS} (4, 11)	9** (5, 15)	5* (2, 9)	56*** (47, 64)
Matched Normals	+ve 95% CI	3 (1, 6)	1 (0, 4)	2 (1, 5)	3 (1, 6)	2 (1, 5)	1 (0, 4)	10 (7, 15)
Specificity %		**97**	**99**	**98**	**97**	**98**	**99**	**90**

Percentage positivity (+ve) with 95% confidence interval (x,y) in each patient group
Panel: autoantibody positivity to any one of the six antigens.
*Denotes p-value relative to normal control results. *p<0.05; **p<0.01; ***p<0.001; NS, not significant p>0.05 (Chi-squared analysis).

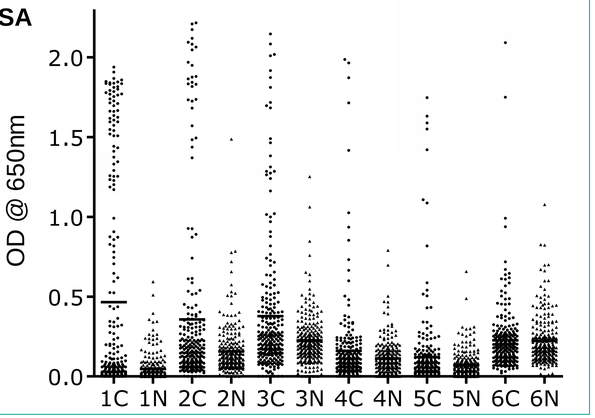
Within the limited disease cohort, 14 patients were confirmed to have Stage 1 disease (five stage 1A). Autoantibodies to p53, SOX2 or HuD were detected in 80% of stage 1A and 50% of the Stage 1 cancers overall.

Notably, four of these stage 1 individuals had samples taken between one and two months before first SCLC diagnosis (due to detection of a paraneoplastic syndrome), and all four had autoantibodies to SOX2.

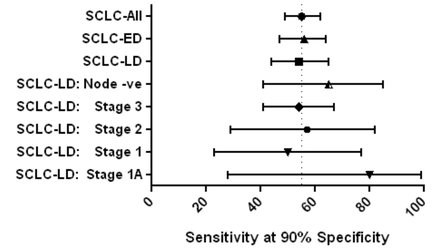
Scatter plots of ELISA Antibody Titres

OD values of individual patients and normal controls to tumor-associated antigens:

1: SOX2
2: HuD
3: p53
4: NY-ESO-1
5: CAGE
6: GBU4-5
C: Cancer sera
N: Normal sera



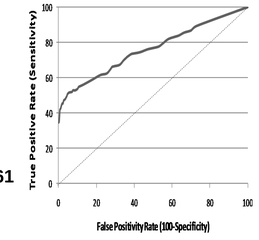
Forest Plot - sensitivity by cancer stage



Receiver Operator Curve

- of the panel For the detection of SCLC

AUC = 0.761 (se 0.027)



CONCLUSIONS

Measuring an autoantibody response to one or more tumour-associated antigens can act as a marker of disease and could provide a sensitive and specific blood test to aid the early detection of SCLC for high-risk individuals.

CLINICAL IMPLICATIONS

The presence of autoantibodies in individuals with SCLC may provide an early indication of disease for high-risk individuals, when the chance of successful treatment would be greatest.

REFERENCES

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