Demonstration Of The Specificity of Human Autoantibody Responses To Tumor-Associated Antigens

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INTRODUCTION

A number of studies have reported the presence of autoantibodies against tumor-associated antigens in lung cancer. Since such autoantibodies may represent an amplification of the early signals of carcinogenesis, an assay has been developed (the *Early*CDT™-Lung test[1,2]) that has shown promise as an aid to the early detection of this devastating disease. The aim of this study was to demonstrate the specificity of the human immune response against the panel of recombinant tumor-associated antigens measured in the *Early*CDT™-Lung test.

METHODS

The six recombinant antigens (p53, SOX2, CAGE, NY-ESO-1, GBU4-5 and Annexin 1) employed in the *Early*CDT™-Lung test were analysed by SDS-PAGE and Western Blotting. The blots were probed both with antigen specific monoclonal antibodies and positive sera. Enzyme Linked Immunosorbant Assays (ELISA) were also performed in which positive sera were pre-incubated with a range of different proteins before being allowed to react with the test antigens in order to show antigen-specific inhibition of binding of these sera.

RESULTS

Western blots showed antigen specific binding of positive sera which was confirmed by the reactivity of monoclonal antibodies. Inhibition ELISAs using positive sera also showed that binding to each antigen could be inhibited by the antigen itself but not VOL (a recombinant antigen produced and purified in the same way as the *Early*CDT™-Lung antigens) or by one of the other cancer antigens in the panel. Calculated IC_{50} values confirmed this (Table 1, below).

Inhibitory protein	Capture Antigen					
	p53	SOX2	CAGE	NY-ESO-1	GBU4-5	Annexin 1
p53	**22.3nM**	**>160nM**	**>160nM**	**>160nM**	**>160nM**	**>160nM**
SOX2	n.d.	**7.2nM**	n.d.	n.d.	n.d.	n.d.
CAGE	n.d.	n.d.	**10.4nM**	n.d.	n.d.	n.d.
NY-ESO-1	**>160nM**	n.d.	n.d.	**1.6nM**	n.d.	n.d.
GBU4-5	n.d.	n.d.	n.d.	n.d.	**46.2nM**	n.d.
Annexin 1	n.d.	n.d.	n.d.	n.d.	n.d.	**147.7nM**
VOL	**>160nM**	**>160nM**	**>160nM**	**>160nM**	**>160nM**	**>160nM**

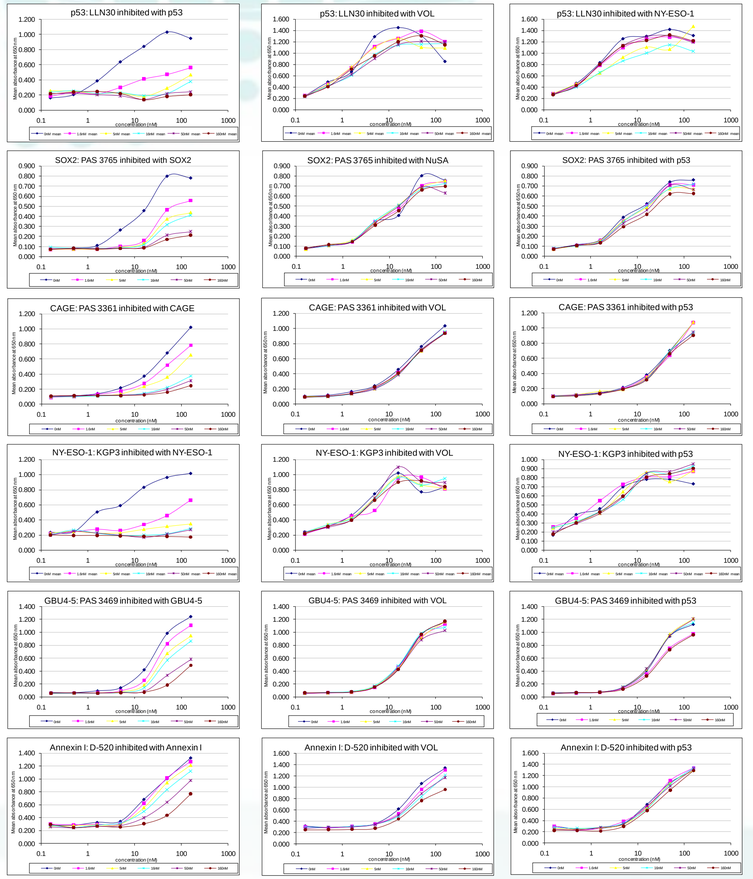

Figure 1: Inhibition of binding of serum autoantibodies to antigens in the *Early*CDT™-Lung panel by the antigen itself (left), a negative control antigen (VOL or NusA) (middle) and an irrelevant antigen (right). IC_{50} measurements are given in Table 1 (left panel).

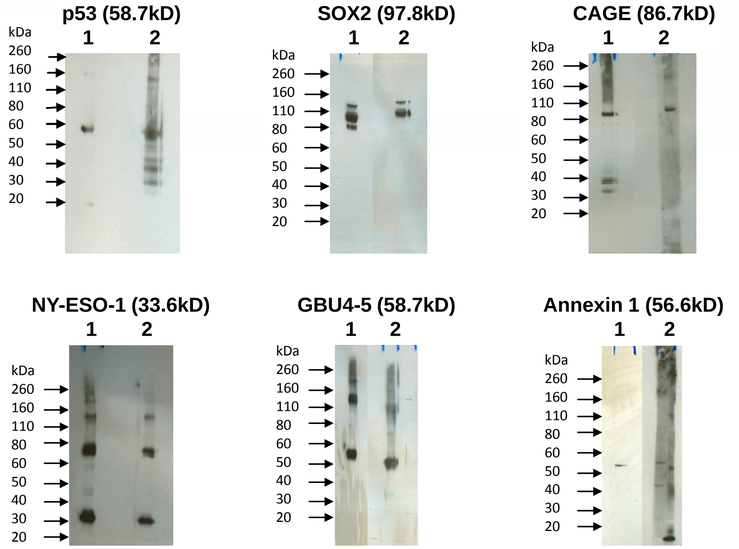

Figure 2: Specificity of autoantibody binding to recombinant tumor-associated antigens as demonstrated by Western Blotting. Binding of an antigen specific monoclonal antibody is shown in lane 1 and binding of an autoantibody positive human serum sample is shown in lane 2 of each blot.

CONCLUSIONS

This study has demonstrated that recombinant proteins derived from bacteria can be used to measure levels of autoantibodies in human serum and that binding of these antibodies is extremely antigen specific.

REFERENCES

1. Murray A, et al. Technical validation of an autoantibody test for lung cancer. *Ann Oncol* 2010; 21:1687-1693.
2. Boyle P, et al. Clinical validation of an autoantibody test for lung cancer. *Ann Oncol*, Epub July 2010; doi: 10.1093/annonc/mdq361.

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