Identification Of Tumor-Associated Autoantibodies To Different Forms Of Recombinant MAGE A4

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BACKGROUND & AIMS

MAGE A4 is a member of the MAGE gene family of cancer/testis antigens and its over-expression has been reported in a number of cancers including lung cancer. Autoantibodies to recombinant forms of this antigen have recently been described[1,2].

E.coli expressed antigens are often aggregated and therefore associated with *E.coli* chaperone proteins following purification via common Nickel affinity purifications[3]. The aim of this project was to determine if further purification of such antigens leads to increased / different immunogenicity in individuals with NSCLC compared to matched normal sera.

METHODS

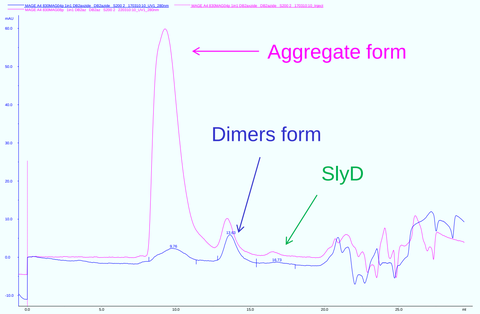
Protein purification:
Specific MAGE A4 cDNA was subcloned, with addition of a small tag, into the pET21b expression vector. BL21(DE3) *E. Coli* competent cells, transformed with pET21-MAGE A4 plasmid, were cultured overnight in LB at 37ºC and used to seed 20L of Power Broth media. Purification of the MAGE A4 protein was by immobilised metal affinity chromatography[3] (IMAC) with (830-MAG-04p) and without (830-MAG-08p) a further size-exclusion chromatography (SEC) purification step to separate aggregated from less native protein (Figure 1).

Immuno-assay:
Serum from patients with lung cancer (n=122) as well as age, sex and smoking matched normal control sera (n=122), were investigated, by ELISA, in 2 separate studies for the presence of autoantibodies to the MAGE-A4 proteins. The presence of AAbs was evaluated using a semi-automated ELISA method where optical densities (OD) were converted to calibrated reference units (RU). Full assay details are described elsewhere[4].

RESULTS

Protein Purification:

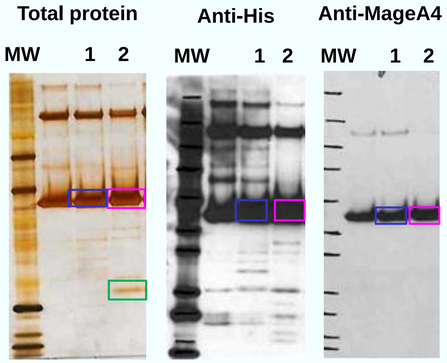
Figure 1:
Analytical SEC analysis Superdex 200 10/300GL column (GE Healthcare) 0.5ml sample injection, run 0.5ml/min on AKTA Purifier (GE Healthcare).
Blue trace: MageA4-BirA 830-MAG-04p purified by quantitative SEC after IMAC (21mg 3.5% recovery)
Pink trace: MageA4-BirA 830-MAG-08p purified only by IMAC, no quantitative SEC (543mg)



Further purification of the MAGE A4 protein resulted in separation of aggregated material from dimeric forms of the antigen, and resulted in the production of a purer protein.

Figure 2:
Silver-staining and Western-blots SDS-PAGE analysis of both MageA4-BirA samples

1. MageA4-BirA 830-MAG-04p SEC purified dimers
2. MageA4-BirA 830-MAG-08p no SEC
SlyD bacterial co-contaminant



Immuno-assay:
ELISAs revealed an increase in sensitivity for the native dimers compared to the aggregated form of MageA4 (Table 1), but when inserted in a panel of 7 antigens (Table 2) the overall positivity did not show such a difference, as some positive samples were also detected by other antigens in the panel.

Table 1: Comparison of MageA4 dimers versus MageA4 aggregates

Comparison study MageA4	Sensitivity (%)	Specificity (%)
830-MAG-04p MageA4 dimers	13 (10.7)	4 (96.7)
830-MAG-08p MageA4 aggregates	7 (5.7)	3 (97.5)

Lung cancer patients (n=122), age, sex and smoking matched normal control sera (n=122)

Table 2: Comparison of MageA4 dimers versus MageA4 aggregates in a 7 antigens panel

Comparison study Panel of 7 antigens	Sensitivity (%)	Specificity (%)
830-MAG-04p MageA4 dimers	51 (41.8)	9 (92.6)
830-MAG-08p MageA4 aggregates	49 (40.2)	9 (92.6)

Lung cancer patients (n=122), age, sex and smoking matched normal control sera (n=122).

Panel of 7 antigens: p53, HuD, SOX2, NY-ESO-1, GBU4-5, CAGE, MAGE A4.

CONCLUSIONS

Autoantibodies to recombinant MAGE A4 are present at a higher frequency in individuals with lung cancer compared to normal controls. Further purification of such antigen may increase the sensitivity for cancer detection in an ELISA, highlighting that purity and composition of proteins may be important in detecting autoantibodies. However, protein recovery after further purification was only 3.5%, therefore pursuit of extensive purity needs to be analysed to measure the potential benefits of increase sensitivity.

REFERENCES

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