Paraneoplastic antigen Ma2 autoantibodies as a blood biomarker for diagnosis, prognosis and detection of early recurrence of small intestine neuroendocrine tumors

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Conclusion

We show that high Ma2 autoantibody titers in the blood of small intestine neuroendocrinet tumor (SI-NET) patients is a sensitive and specific biomarker, superior to chromogranin A (CgA) for the risk of recurrence after radical operation of these tumors.

Background

Small intestine neuroendocrine tumors (SI-NETs) belong to a rare group of cancer. Most patients have developed metastatic disease at the time of diagnosis, for which there is currently no cure. The delay in diagnosis is a major issue in the clinical management of the patients and new markers are urgently needed. We have previously identified paraneoplastic antigen Ma2 (PNMA2) as a novel SI-NET tissue biomarker. Ma2 autoantibodies are often present in sera from cancer patients.

Aims

Are Ma2 autoantibodies also detectable in blood of SI-NET patients?

Are circulating anti-Ma2 a novel potential diagnostic and/or prognostic biomarker?

Can anti-Ma2 be detected in other types of NETs such as Lung NETs?

Figure 1. Immunostaining of Ma2 on specimens from untreated SI-NET patients. The figure shows four representative staining from two out six patients with primary tumors and corresponding lymph node or liver metatasis. Bar=50μm.

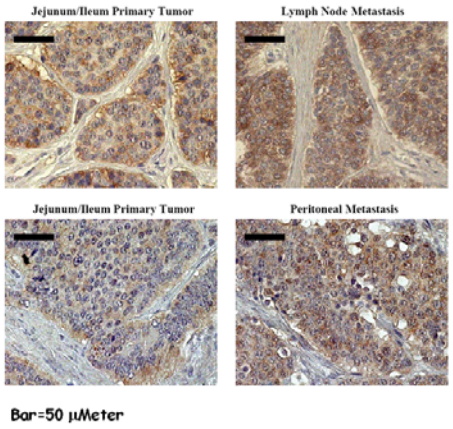
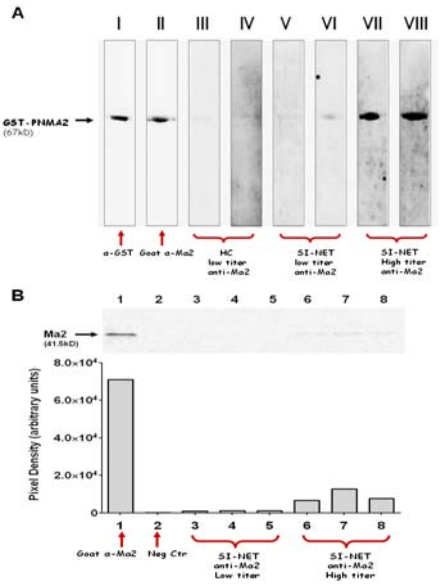


Figure 3. Detection of Ma2 autoantibodies in serum of healthy controls and primary SI-NET patients. A) GST-tagged PNMA2 recombinant protein was subjected to Western blotting analysis. Lanes III-IV, sera from 2 healthy donors; Lanes V-VI sera from 2 primary SI-NET patients with low anti-Ma2 titers; Lanes VII-VIII, sera from 2 primary SI-NET patients with high anti-Ma2 titers. Lanes III to VIII show differences in the amount of Ma2 autoantibodies. B) autoradiographic image and semiquantitative measurement of sequentially immunoprecipitated 35S-Met-Ma2



Results

The novel Ma2 autoantibody ELISA showed high sensitivity, specificity and accuracy. We observed that SI-NET patients expressing Ma2 autoantibody levels below the cutoff had a longer progression and recurrence free survival compared to those with higher titers. We also detected higher levels of Ma2 autoantibodies in blood samples from TLC and ALC patients than from healthy controls, as previously shown in small cell lung carcinoma samples.

Figure 2. A novel indirect ELISA detects Ma2 autoantibodies in serum from SI-NET patients. Healthy controls (HC), untreated SI-NET patients with primary tumor (P), lymph node metastasis (LNM) and liver metastasis (LM) were evaluated for the presence of Ma2 autoantibodies. Horizontal lines indicate the cutoff at 1.96 SD above the mean of anti-Ma2 concentration of HC. Significant differences were detected between HC and each stage of malignancy (A). ROC analyses and AUCs results are shown in B, C and D.

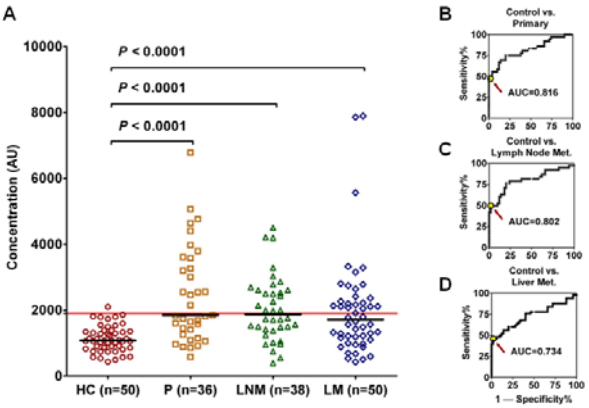
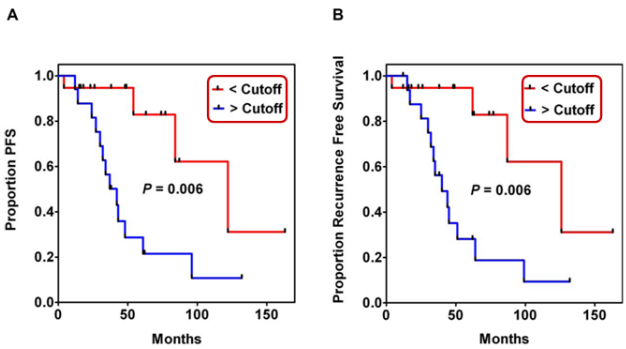


Figure 4. PFS and RFS of primary SI-NET patients after surgery with curative intent depend on Ma2 autoantibody titers. Patients were divided in two groups based on the Ma2 autoantibody titers either below or above the cutoff at 1900 AU as described in Material and Methods. Kaplan-Meier survival curve analyses were plotted for PFS (A) and RFS (B). The p-values of the differences between the two groups were obtained by using the log-rank test for each evaluation.



Methods

A novel indirect enzyme-linked immunosorbent assay (ELISA) was set up to detect Ma2 autoantibodies in blood samples of patients with SI-NET at different stages of disease. In total, 124 blood samples of SI-NET patients were included in the study. Ma2 autoantibodies in the blood from SI-NET patients were verified by western blot and sequential immunoprecipitation.