COMMENTARY

SEREX: a promising approach for identification of thyroid cancer serological biomarkers

Mario Vitale

Department of Medicine and Surgery, University of Salerno, Salerno, Italy

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A relevant clinical issue is to distinguish the very frequent thyroid benign nodules from rare malignant tumours. Unfortunately, fine-needle aspiration cytology (FNAC), the primary means for distinguishing between benign and malignant nodules, often yields inconclusive results[1]. In the last decades, a large number of studies have been performed in the attempt to find molecular markers useful for the preoperative diagnosis of thyroid nodules. Most of these markers are tumour-expressed proteins (galectin-3, HBME-1, cytokeratin-19) or genes (*BRAF*^{V600E}, *RET/PTC, PAX-8/PPR*γ) that can be detected by immunohistological methods or by genetic analysis on biopsy specimens.[2–4] Particularly in areas with low iodine intake where goitre is more frequently multinodular, there is the issue of which nodule/s should be analysed by FNAC, because the analysis of all nodules is not always feasible or appropriate. The availability of a serological diagnostic method to detect the presence of a thyroid cancer would be very useful and highly desirable. Cancer is developed and sustained by genetic abnormalities that produce mutated or aberrantly expressed proteins. Mutated proteins and normal proteins inappropriately expressed may evoke a spontaneous immune response. Cellular immunity and antibody production against tumour molecules are defence tools of the host, whose efficacy against cancer development and progression is still to be fully understood. Spontaneous antibodies against tumour antigens are commonly produced and detectable in the serum of a large number of patients with cancer. Spontaneous antitumour antibodies can be evoked by mutated proteins present exclusively in tumours (tumour-specific antigens). More frequently, autoantibodies are evoked by aberrantly expressed proteins (tumour-associated antigens, TAAs) that become immunogenic because of their inappropriate expression. Some of these TAAs are molecules expressed during the ontogenesis and, thus in the postfoetal age, are present solely in the testis. For this reason, these antigens are named cancer–testis antigens (CTAs). TAA identification can be achieved by SEREX (serological analysis of recombinant cDNA expression libraries). The SEREX method was originally developed by Michael Pfreundschuh and colleagues in 1995.[5] Almost 3000 TAAs have been identified by this method and are listed in the Cancer Immunome Database (http://ludwig-sun5.unil.ch/CancerImmunomeDB) developed by the Ludwig Institute of Cancer Research. These autoantibodies can be used as biomarkers for the diagnosis and prognosis of cancer and as serological markers of disease recurrence. The inconstant and dynamic nature of cancer makes a diagnostic test based on a single TAA inadequate. However, the lack of adequate sensitivity and specificity offered by a single TAA may be overcome by using a panel of TAAs where multiple autoantibodies are detected simultaneously. Detection of early-stage breast cancer by three TAA autoantibodies reached 77·0% sensitivity and 82·8% specificity.[6] A five TAAs-based test was able to discriminate between patients with early colorectal carcinoma and healthy controls, with sensitivities of 90·0% and specificities of 91·7%.[7] While TAA screening and search for the corresponding autoantibodies have been conducted for breast, prostate, lung, colon and many other cancers, very little has been conducted for thyroid cancer. The expression analysis of 13 selected CTAs demonstrated the presence of NY-ESO-1 in about 60% of sporadic medullary thyroid carcinomas and the presence of the corresponding antibody in 36% of patients.[8] Few other data on thyroid cancer have been obtained as complementary results in studies investigating other cancer types.

In this background, the study by Izawa S. *et al.* is one of the few reports about serological screening for TAA by SEREX in patients with thyroid cancer.[9,10] The authors screened a cDNA library of an undifferentiated thyroid cancer with autologous serum, identifying the presence of antibodies against WD repeat domain 1 (WDR1) and fibronectin 1. Although it is disappointing that only two TAAs have been found, this study demonstrates that this approach is feasible and promising. Microenvironment, including extracellular matrix, adhesion molecules and stromal cells, is crucial in thyroid cancer progression and spreading.[11–13] While it is possible that host immunoresponse against thyroid cancer is blunted by a distinguishing microenvironment, a broader methodological approach such as testis cDNA immunoscreening with sera from multiple patients might disclose more antigens associated with advanced thyroid cancer and corresponding autoanti-

Correspondence: Mario Vitale, Department of Medicine and Surgery, University of Salerno, Baronissi 84081, Salerno, Italy.
Tel.: 0039089672539;
E-mail: mavitale@unisa.it

bodies. The study highlights the value of anti-WDR1 antibodies in a serological test to screen for thyroid advanced cancer. However, more useful would be a serological test for early detection of thyroid cancer, and importantly, the detection of those microcarcinomas that for still unknown reasons is aggressive.[14] Such a test would address subjects with nodular goitre to a more accurate conventional evaluation by FNAC. A more extensive screening of cDNA libraries of normal testis and different tumours with multiple patient sera could identify a number of immunogenic TAAs sufficient to produce a highly accurate diagnostic test. Serological screening would be a simple, cost-effective and noninvasive method to be used in a risk population. The identification of ectopic proteins in tumours by SEREX may be helpful also to uncover new molecular mechanisms of tumour development or progression. WDR1 is a protein presumed to induce the disassembly of actin filaments, and thus, it could have a role in tumour spreading. WDR1 and other immunogenic TAAs that will be identified by SEREX in advanced thyroid carcinomas might be useful also for therapeutic purposes. Patients who develop radioactive iodine –refractory thyroid carcinoma have few treatment options. Immunogenic TAAs identified by SEREX may be used as targets for immunotherapy.[15] Direct TAA immunization and dendritic cell-based tumour vaccines are promising strategies to stimulate immune responses against malignant cells.[16] The immunological approach to cancer provides the opportunity to new methods for early detection of the disease but also can stimulate the antitumour immune response in patients with cancer. This is a completely unexplored field of research for thyroid cancer.

References

1 Gharib, H. & Goellner, J.R. (1993) Fine-needle aspiration biopsy of the thyroid: an appraisal. *Annals of Internal Medicine*, **118**, 282–289.

2 Sapio, M.R., Guerra, A., Posca, D. *et al.* (2007) Combined analysis of galectin-3 and BRAFV600E improves the accuracy of fine-needle aspiration biopsy with cytological findings suspicious for papillary thyroid carcinoma. *Endocrine-Related Cancer*, **14**, 1089–1097.

3 Guerra, A., Sapio, M.R., Marotta, V. *et al.* (2011) Prevalence of RET/PTC rearrangement in benign and malignant thyroid nodules and its clinical application. *Endocrine Journal*, **58**, 31–38.

4 Guerra, A., Fugazzola, L., Marotta, V. *et al.* (2012) A high percentage of BRAFV600E alleles in papillary thyroid carcinoma predicts a poorer outcome. *Journal of Clinical Endocrinology and Metabolism*, **97**, 2333–2340.

5 Sahin, U., Tureci, O., Schmitt, H. *et al.* (1995) Human neoplasms elicit multiple specific immune responses in the autologous host. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 11810–11813.

6 Zhong, L., Ge, K., Zu, J.C. *et al.* (2008) Autoantibodies as potential biomarkers for breast cancer. *Breast Cancer Research*, **10**, R40.

7 Chang, W., Wu, L., Cao, F. *et al.* (2011) Development of autoantibody signatures as biomarkers for early detection of colorectal carcinoma. *Clinical Cancer Research*, **17**, 5715–5724.

8 Maio, M., Coral, S., Sigalotti, L. *et al.* (2003) Analysis of cancer/testis antigens in sporadic medullary thyroid carcinoma: expression and humoral response to NY-ESO-1. *Journal of Clinical Endocrinology and Metabolism*, **88**, 748–754.

9 Kiyamova, R., Garifulin, O., Gryshkova, V. *et al.* (2012) Preliminary study of thyroid and colon cancers-associated antigens and their cognate autoantibodies as potential cancer biomarkers. *Biomarkers*, **17**, 362–371.

10 Izawa, S., Okamura, T., Matsuzawa, K. *et al.* (in press) Autoantibody against WD repeat domain 1 is a novel serological biomarker for screening of thyroid neoplasm. *Clinical Endocrinology (Oxford)*, in press.

11 Nucera, C., Lawler, J. & Parangi, S. (2011) BRAF(V600E) and microenvironment in thyroid cancer: a functional link to drive cancer progression. *Cancer Research*, **71**, 2417–2422.

12 Vitale, M., Bassi, V., Illario, M. *et al.* (1994) Loss of polarity and de novo expression of the beta 1 family of integrins in thyroid tumors. *International Journal of Cancer*, **59**, 185–190.

13 Di Matola, T., Mueller, F., Fenzi, G. *et al.* (2000) Serum withdrawal-induced apoptosis in thyroid cells is caused by loss of fibronectin-integrin interaction. *Journal of Clinical Endocrinology and Metabolism*, **85**, 1188–1193.

14 Nucera, C., Lawler, J., Hodin, R. *et al.* (2010) The BRAFV600E mutation: what is it really orchestrating in thyroid cancer? *Oncotarget*, **1**, 751–756.

15 Chen, Y.T. & Old, L.J. (1999) Cancer-testis antigens: targets for cancer immunotherapy. *The Cancer Journal from Scientific American*, **5**, 16–17.

16 Banchereau, J. & Steinman, R.M. (1998) Dendritic cells and the control of immunity. *Nature*, **392**, 245–252.