Harnessing immunity for cancer marker discovery

The display of a random peptide library on the surface of phage has been applied to the discovery of antigens specific to prostate cancer.

Sam Hanash

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The identification of tumor antigens that elicit an antibody-based immune response may be useful in cancer screening and immunotherapy. Several approaches are being developed to detect such tumor antigens. In this issue, Mintz *et al.*[1] report on the implementation of a screening method, which they have applied to prostate cancer, that is based on random peptide library phage display to select peptides recognized by circulating antibodies in the sera of cancer patients (Fig. 1).

It is somewhat paradoxical that the spectacular achievements made in the past two decades in defining molecular genetic alterations in cancer have not translated either into effective strategies for screening and early diagnosis or, with some exceptions, into effective therapies. However, one senses that as we broaden our focus from the delineation of specific genetic alterations to the implementation of a systems-pathology approach to cancer, numerous leads will emerge that will have an impact on cancer diagnosis and treatment.

A systems-pathology approach to cancer seeks an understanding of how genomic alterations, together with host factors, affect gene expression and ultimately tumor behavior. A major host factor is the immune system[2]. There is increasing evidence for an immune response to cancer in humans, as demonstrated in part by the identification of autoantibodies against a number of intracellular and surface antigens detectable in sera from patients with different cancer types.

Clearly, tumors may develop in the presence of this antibody-mediated immune response. However, the identification of a panel of antigenic markers that are tumor specific and that elicit immunoreactivity early in tumor development and at a high frequency would provide an effective strategy for cancer screening. The race is on to develop such a panel. Harnessing the immune response to identify novel cancer biomarkers is an attractive strategy, because the immune system performs biological amplification that is the equivalent of a PCR reaction by generating a detectable signal, with antigenic tumor proteins as templates, beginning at an early stage during tumor development when the tumor may be otherwise undetectable.

In this issue, Mintz *et al.* describe their identification of a consensus motif by selective binding to antibodies in the serum of prostate cancer patients compared with serum from blood donors. The corresponding protein eliciting the immune response is identified by mass spectrometry analysis as glucose-regulated protein–78 kDa (GRP78), a member of the heat-shock protein family. The findings provide a proof of principle that a strategy based on a random peptide library and peptide mimicry can uncover targets of antibodies in patient sera.

Although identification of GRP78 antigen provides validation of the phage-display approach, the GRP78 protein is fairly ubiquitous, and clearly GRP78

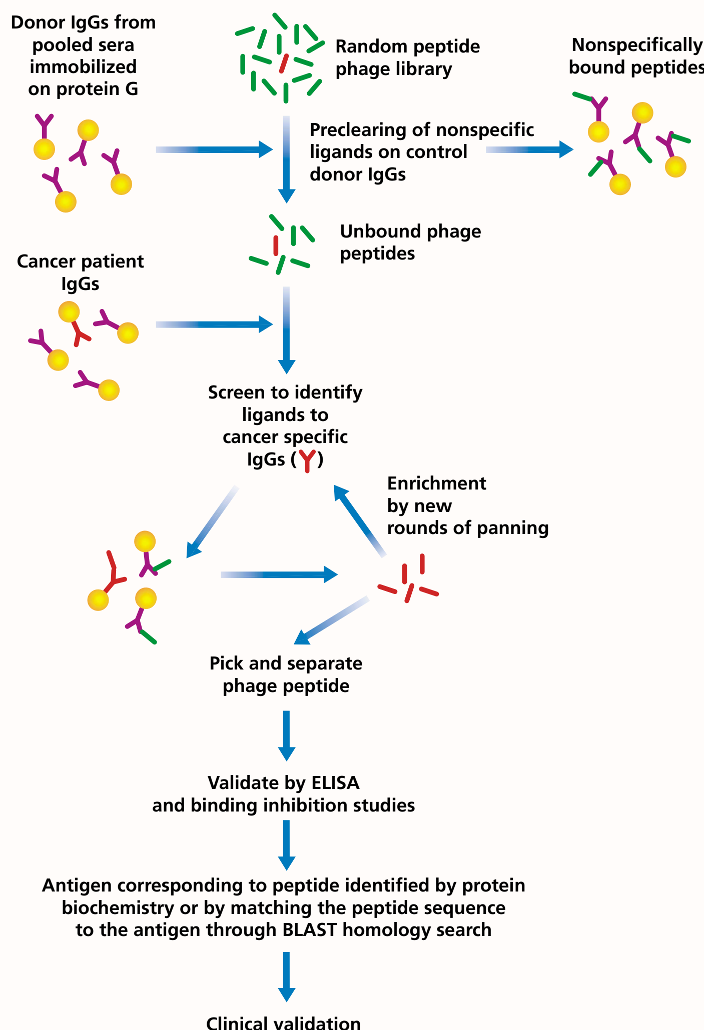

Figure 1. Strategy for tumor antigen identification using random peptide library phage display.

Sam Hanash is a professor in the Department of Pediatrics at the University of Michigan Medical Center, Ann Arbor, MI 48109 (shanash@umich.edu).

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autoantibodies occur late during tumor progression. Therefore, as a screening test for cancer, detection of GRP78 antibodies is likely to be of only limited utility. Yet if the strategy leads to the identification of additional antigenic proteins and provides a molecular fingerprint for prostate cancer, then the use of random peptide libraries would be quite appealing. One hopes that antigens that induce an antibody response in prostate cancer early in the course of tumor development would be a part of this molecular fingerprint.

What are the alternative strategies available for deciphering the repertoire of antibodies in sera of cancer patients? A large number of antigenic targets have been identified by screening expression libraries with patient sera[3,4]. However, most of these antigens elicit antibodies in only a relatively small proportion of patients, which reduces their utility. Proteomics has provided an alternative approach to expression-library screening and is increasingly relied upon for the identification of tumor antigens. The merit of proteomics in this regard is that it allows analysis of proteins and peptides in their post-transcriptionally modified states, as they occur in cancer cells. These modifications, such as glycosylation, may be immunogenic, and proteomics technologies may preserve such epitopes.

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The phage-display approach joins several proteomics strategies now available for identifying tumor antigens. In a recent elegant example, human leukocyte antigen (HLA)-restricted tumor-specific antigens were identified by transfecting human breast, ovarian, and prostate tumor cell lines with truncated versions of the genes encoding HLA-A2 and HLA-B7 (ref. 5). Soluble HLA secreted by the cell lines was purified and analyzed by mass spectrometry, yielding a large pool of peptides. The pool included novel candidates for tumor antigens as well as established antigens, such as MAGE-B2 and mucin.

The standard proteomic tools of two-dimensional gels and mass spectrometry have uncovered a repertoire of antigens that, curiously, does not overlap much with antigens uncovered using expression library–based approaches[6]. With two-dimensional gels, several thousand cellular proteins from tumor tissue or tumor cell lines are separated and transferred onto membranes that are then incubated with subject sera. Proteins that react specifically with antibodies in sera from cancer patients are identified by mass spectrometry and further evaluated with respect to their specificity.

In a study of lung cancer, sera from 60% of patients with lung adenocarcinoma, but none of the cancer-free controls, exhibited immunoglobulin G–based reactivity against proteins identified as glycosylated annexins I and II[7]. Anti-annexin antibodies were detected not only in early-stage lung cancer but also, in a blinded pilot study carried out before diagnosis, in patients later found to have cancer (S.H., unpublished data). For most antigenic proteins that induce an antibody response in cancer and that have been identified using the proteomic approach, a post-translational modification—in some cases, one that is tumor specific[8]—contributed to the immune response.

Microarrays containing the repertoire of proteins expressed in tumor cells should substantially accelerate the pace of discovery of tumor antigens and could provide a molecular signature for immune responses in different types of cancer[9]. Numerous sources of cancer biomarkers other than tumor antigens are being pursued. These include detection of genomic alterations that are quantifiable in serum and biological fluids, comparative analysis of gene expression in normal and tumor tissues to identify aberrantly expressed proteins that may represent novel markers, analysis of secreted proteins in cell lines and primary cultures, and comparative analysis of serum protein patterns[10]. One can hope that this impressive tool set for cancer biomarker discovery will fulfill its potential and provide more effective means for cancer screening and early diagnosis.

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