HEPA and PARSE
Systematic discovery
of clinically relevant tumor-specific antigens

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The effective discovery of tumor-specific antigens (TSAs) holds the key for the development of new diagnostic assays and immunotherapeutic approaches against cancer. Here, we discuss our recently developed technologies, HEPA and PARSE, which allow for the systematic identification of TSAs, generating a reservoir of immunologically and clinically relevant targets.

In recent years, cancer immunodiagnostic tools that detect tumor-specific antigens (TSAs) have been widely integrated into the clinical practice.[1] For example, α fetoprotein (AFP), CA19-9, CA125, CA15-3, carcinoembryonic antigen (CEA) and prostate-specific antigen (PSA) detection kits are commonly used in the clinic for the early detection or monitoring of a variety of human cancers. In addition, some immunotherapies targeting tumor-specific antigens (TSAs) have been incorporated into the clinical routine. Provenge, a therapeutic cancer vaccine targeting the prostatic acid phosphatase (ACPP), has been shown to prolong the survival of subjects bearing metastatic hormone-refractory prostate cancer,[2] and has been approved by the US FDA for use in this group of patients.

The effective discovery of clinically relevant TSAs holds a fundamental role for the development of new diagnostic tools and anticancer (immuno)therapies.

Beyond traditional methods, bioinformatics has been largely exploited for the discovery of novel TSAs.[3] The significant progress of genome-scale profiling technologies, including microarray- and next generation sequencing-based approaches, has greatly improved our ability to explore the cancer genome. By using comprehensive bioinformatic methods, we have identified *UCA1*, a novel non-coding RNA gene, as a marker for bladder cancer.[4] Without doubt, the systematic exploration of cancer-specific antigen-coding genome will help us to better understand the behavior of tumor cells, and to add new candidates to the ever expanding list of TSAs that may be exploited for the generation of diagnostic and immunotherapeutic tools.

To achieve this goal, we developed Heterogeneous Expression Profile Analysis (HEPA),[5] a new computational technology that highlights the genes that are heterogeneously overexpressed by cancer cells as compared with their normal counterparts (**Fig. 1**). Rather than relying on traditional statistical tests, HEPA computationally describes the unique expression patterns of established clinically relevant TSAs (**Fig. 1A**) by virtue of an innovative mathematical scheme (**Fig. 1B**). By using HEPA, we are able to isolate TSA-coding genes that have had previously been shown to be clinically relevant (including most prototypic TSAs) directly from the human genome, which comprises tens of thousands of genes. This demonstrates the robustness of the HEPA score in prioritizing clinically important TSAs. HEPA indeed greatly improved the predictive power (measured in term of tumor specificity) of statistical methods that we had previously developed in the Human Potential Tumor Associated Antigen database.[6]

Accompanying HEPA, we developed Protein A/G based Reverse Serological Evaluation (PARSE) (**Fig. 1C**), a novel
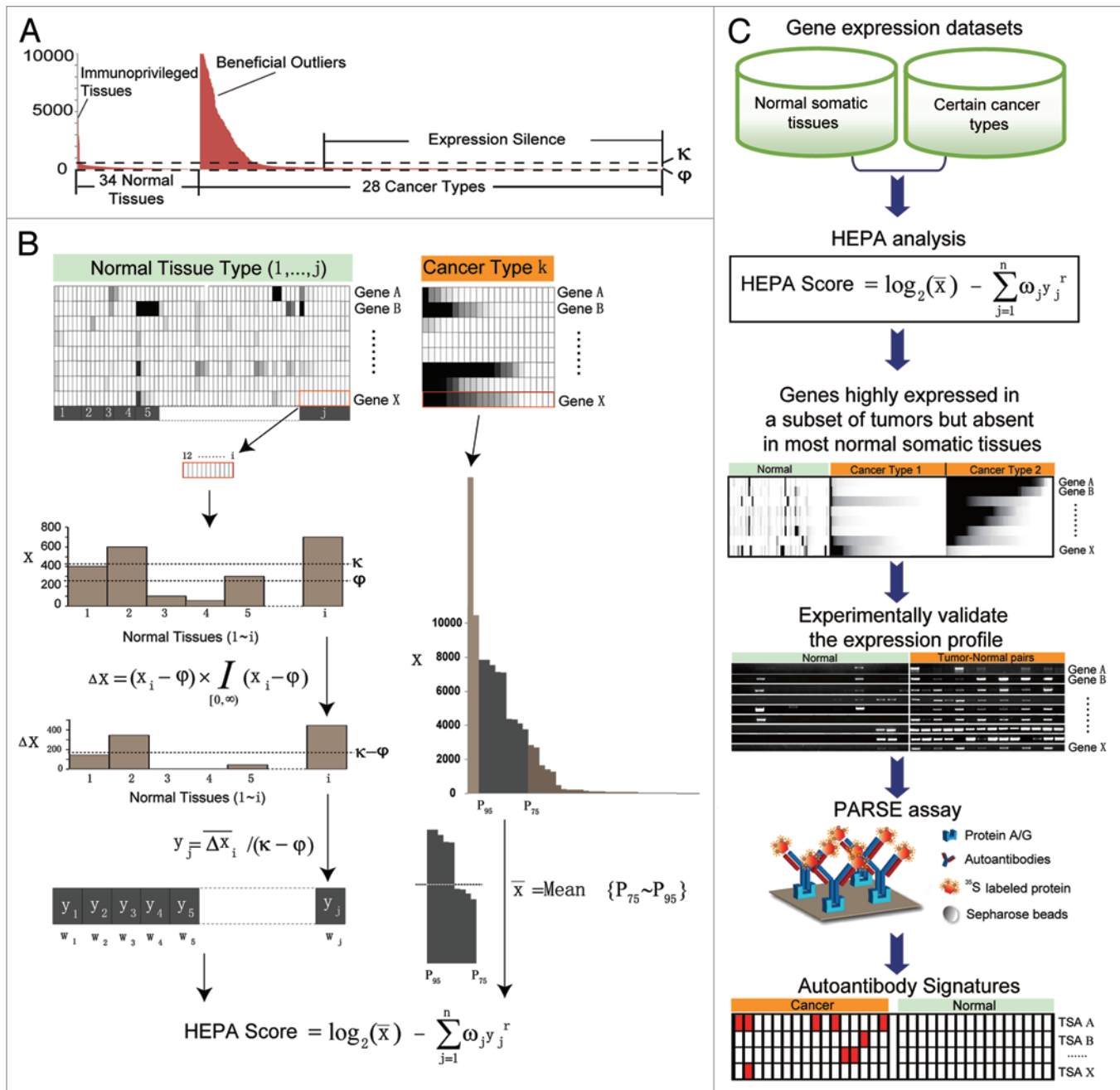
Figure 1. The HEPA-PARSE technology. (**A**) The heterogeneous expression profile of MAGE-A3, a canonical cancer-testis antigen. (**B**) Algorithms underlying the HEPA technology. (**C**) Flowchart of the integrated HEPA-PARSE technology in the discovery of autoantibody signatures in cancer. Figure adapted from reference 5, with permissions.

assay to rapidly detect circulating autoantibodies against putative TSAs. PARSE leverages the mammalian in vitro translation system as an efficient way to produce TSAs in a relative natural conformation (including post-translational modifications), which are used to capture autoantibodies from patient sera. This assay presumably generates a precise picture of autoantibody responses in cancer patients by detecting the full spectrum of natural epitopes. Remarkably, tumor-specific autoantibody responses against seven candidate targets were detected by PARSE in 4% to 11% of patients, indicating that these TSAs are capable of inducing spontaneous immune responses in vivo. Such autoantibody signatures were distinct in patients affected by lung and stomach cancers.[5] Importantly, the interrogation of an independent cohort of 149 patients and 123 healthy donors validated the predictive value of the autoantibody signature in the setting of lung cancer.

Understanding the mutual interactions between tumor cells and the host also constitutes a critical issue for the development of novel anticancer (immune)therapies. For a long time, it has been proposed that TSAs would play a critical role during

gametogenesis or embryonic development, and that the ectopic expression of such proteins in adult cells would promote malignancy. As the functions of most TSAs are still unknown, a mysterious gate has just started to open.

PRAME, a TSA predominantly expressed by human melanomas, has been shown to constitute a dominant repressor of retinoic acid receptor (RAR)-conveyed signals.[7] Hence, PRAME overexpression is frequently associated with growth or survival advantages by virtue of its ability to antagonize RAR signaling. Additionally, Melanoma-associated antigens (MAGEs), constituting a large family of TSAs with unknown function, are expressed in multiple types of tumors. MAGEs have recently been shown to interact with—and hence promote the activity of—RING ubiquitin ligases.[8] These studies and others of the same kind that will undoubtedly follow are critical as they provide mechanistic details and new insights into the role of TSAs in the etiology of cancer.

These observations strongly indicate that TSA are not simply a "by-product" of malignant transformation but rather exert oncogenic properties and facilitate oncogenesis and tumor progression. By using the HEPA-PARSE technology, we identified a panel of TSAs with remarkable unique tumor expression patterns. Previous reports indicated that several of them have important physiological functions, while their role in cancer remains unknown.

IQGAP3 is a novel member in the IQGAP family. Although the founding member of the family (i.e., IQGAP1) has been implicated in diverse signaling pathways related to tumorigenesis, the biological role of IQGAP3 is still poorly defined. Recently, IQGAP3 has been suggested to regulate cell proliferation via the RAS-dependent activation of extracellular signal-regulated kinase (ERK).[9] Vestigial-like 1 (VGLL1), another TSA of our list, represents cancer-placenta (CP) antigens. VGLL1 encodes a transcriptional co-activator that binds to TEA domain-containing transcription factors. Recent studies have indeed shown that VGLL1 interacts with the transcription factor TEAD to facilitates cell proliferation, presumably promoting tumor progression.[10]

It should be emphasized that - besides the identification of novel TSAs - with adjusted parameters, the HEPA technology can also be used to identify overexpressed antigens that may be important in the therapeutic setting, such as the epidermal growth factor receptor (EGFR) and HER2. Furthermore, HEPA can be easily adapted to other applications, such as the identification of biomarkers, predictive autoantibody signatures, vaccine targets, as well as membrane-associated antigens, a process that could be further enhanced by integrating HEPA with subcellular localization and proteomic data sets. With the development of the RNA deep sequencing technology, we expect that the interest in the HEPA technology as an approach to interrogate these new data sets will increase as well.

In conclusion, our studies established an integrated computational-experimental technology to uncover the cancer-specific antigen-coding genome, providing a reservoir of immunologically and clinically relevant targets. This integrated approach will not only generate novel insights into the cancer-specific molecular portrait, but also promote the development of novel diagnostic and (immune)therapeutic tools.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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