AUTHOR’S VIEW

Non-invasive glioblastoma immunoprofiling by printed peptide arrays

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ABSTRACT

Immune monitoring assays for patient stratification and treatment efficacy in clinical trials are in demand. We have recently described a cost-effective non-invasive assay to determine the immune status of glioblastoma patients. Profiling antitumor serum antibodies by customized printed peptide arrays identified response against a tenascin-C (TNC) peptide as a robust prognostic biomarker.

It is increasingly accepted that the immune system actively contributes to the emergence, editing and progression of glioblastomas (GBMs). This pivotal insight has risen hope that immunotherapy, if effectively applied, could become a powerful treatment option alongside maximal safe resection and radiochemotherapy. However, experiences in other tumor entities have indicated that immunotherapy might not be a magic bullet for every patient. Choosing the right patient subgroups (i.e. patient stratification) was often key to the success of clinical trials interrogating the benefit of immunotherapy. In addition, without a profound understanding of tumor-specific immune responses, finding informative clinical endpoints is proving difficult. As a consequence, there is a need for meaningful immune assays to profile and monitor the immune status of tumor patients. As for GBMs, antitumor T-cell responses were described, but are still challenging to quantify in a timely and high-throughput manner. On the contrary, little is known about antitumor B-cell response in GBM, but serum antibodies could be observed and are comparatively easy to quantify.

In our study published in Oncotarget, we aimed at developing a non-invasive assay to determine the immune response of glioblastoma patients against selected antigens by profiling of serum antibodies (for graphical abstract of study design see Fig. 1). To this end, we relied on peptide microarrays as they allow for the multiplex analysis of antibody responses against several thousand of peptides at the same time while requiring a minimal sample volume. We employed the novel PEPperCHIP* (PEPperPRINT GmBH) peptide microarray technology offering a highly customizable array design by on-chip combinatorial synthesis with a modified laser printer. Validity of the technology for our application could be confirmed by replication of parts of the analysis (n = 129) with an independent peptide microarray technology (pre-synthesized peptides spotted on an array).

Antigen and peptide selection was crucial for identifying clinically meaningful antibody responses, as it is unfeasible to cover the complete linear proteome using peptide microarrays. The peptide array of our training study covered the linear amino acid sequence of six tumor-associated antigens (TAAs) discovered in glioblastoma as 1745 overlapping 13-mere peptides: epidermal growth factor receptor (EGFR), TNC, fatty acid binding protein 5 (FABP5), melanoma-associated antigen 3 (MAGEA3), glioma-expressed antigen 2 (GLEA2) and PHD finger protein 3 (PHF3). TAAs are known to be targets of both humoral and cell-mediated immune response as they primarily embody (i) re-expressed proteins of embryonic development, (ii) markedly overexpressed proteins upon tumorigenesis and (iii) proteins with a change in amino acid sequence (neoantigens). Our strategy to identify prognostic antibody responses was to compare the antibody profile against this 1745-peptide array in 10 long-term (LTS; >36 months) and 14 short-term surviving (STS; 6–10 months) glioblastoma patients.

Designing a new array with the top 30 peptides of differential antibody response in glioblastoma patients with an extremely opposite survival (LTS vs. STS) led to a considerable decrease in cost, sample volume (from 10 μL to 1 μL) and ultimately ensured a timely high-throughput analysis in subsequent validation studies. Avoiding overfitting and reducing the number of false-positive results, we validated our top 30 array in two independent multi-center study sets (n = 61 and n = 129). Moreover, our study was exceptional as all 190 patients exclusively consisted of primary IDH1-wildtype GBM patients. This is of major importance because increasing evidence suggests that IDH1-mutant GBMs have been heavily confounding...
previous biomarker studies, as they more closely resemble GBMs arising from lower-grade lesions (secondary GBMs) and have a significantly better outcome. This stringent approach led to the identification of an increased titer against the TNC peptide VCEDGFTGPDCAE as a prognostic biomarker in both study sets. The predictive performance was independent of known prognostic factors (age, Karnofsky Performance Index and MGMT promoter methylation). The importance of multivariate regression analysis for biomarker development cannot be overstated.

A common shortfall of peptide microarray analyses is the lack of statistical approaches to correct for the marked difference in sensitivity between different fluorescence measurements. Normalization procedures developed for DNA microarrays (e.g. quantile normalization) are not directly applicable to protein microarrays, as they exert the majority of spots that are not differentially expressed between arrays. We bypassed this problem by using a relative rank-based comparison of the antibody titers between patients. This approach enabled us to even compare serum with plasma measurements. However, a comprehensive analysis is needed to systematically assess the difference between antibody measurements in serum and plasma samples.

As a proof of concept for the novel PEPperCHIP® printed peptide array technology, comparing pre-operative levels of antitumor serum antibodies led to a robust prognostic biomarker. We envision that the next promising application of our assay is the non-invasive monitoring of immune responses over the course of the disease. Multiple pre- and post-operative measurements will enable the interrogation of the impact of surgery, radio- and chemotherapy. Likewise, high-frequency measurements will guide an estimation of stochastic noise in the measurement. Due to the extremely low sample volume of only 1 μL (top 30 arrays) we further conceive that it might be synergistic to combine our analysis with the detection of circulating cell-free DNA or other serum-based analyses. Ultimately, further work is needed to elucidate the dynamics of serum antibodies and their immunogenicity.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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