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Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

Patients and tissue selection

The study was approved by the ethics review board of the National Health Group, Singapore. Sixteen formalin-fixed paraffin-embedded (FFPE) blocks containing lesional skin biopsies of patients with Henoch–Schönlein purpura (HSP) from the archives of the Histopathology Laboratory at the National Skin Centre, Singapore, were identified. The patients ($n = 16$; male (M)/ female (F): 7/9; age 14–73 years) were diagnosed between January 2013 and December 2015 and the diagnostic criteria were: (*i*) palpable purpura or petechiae clinically consistent with HSP, (*ii*) skin histology demonstrating leukocytoclastic vasculitis, and (*iii*) IgA deposition in vessel walls on immunohistochemical examination. The biopsies were all obtained before the patients underwent treatment for HSP.

The controls consist of 10 FFPE blocks of normal skin, as well as 11 blocks from patients with an inflammatory skin disease (pustular psoriasis 3, neutrophilic dermatoses 2, bacterial skin infections 6). From each block, 15–20 sections 10 μm thick were cut using a conventional microtome (Leica RM2125); a new sterile blade was used for each block to avoid contamination among the samples.

RNA extraction

All reactions were performed in an RNase-free environment and RNase-free tips and microtubes were used. Total RNA was extracted using RNeasy FFPE Kit from QIAGEN GmbH (Hilden, Germany) according to the manufacturer's instructions. RNA yield was assessed using Quant-iT Ribogreen RNA assay kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) following manufacturer's instruction and measured by Tecan Infinite M200 Monochromator microplate reader (Tecan, Switzerland).

Nanostring analysis

All RNAs were analysed on Agilent Bioanalyzer for quality assessment with RNA Integrity Number (RIN) range from 1.2 to 5.8 and median RIN 2.3. Direct mRNA expression levels of the samples were measured using the NanoString nCounter gene expression system. Total RNA of 11–100 ng were hybridized with probes from the nCounter Human Immunology v2 panel (NanoString Technologies, Seattle, WA, USA) at 65°C for 19 h according to the nCounter™ Gene Expression Assay Manual. Excess probes were washed away using a 2-step magnetic bead-based purification on the nCounter™ Prep Station (GEN1). The nCounter™ Digital Analyzer (GEN1) was used to count individual fluorescent barcodes and quantify target molecules present in each sample. For each assay, a high-density scan (600 fields of view) was performed.

Nanostring data were extracted as RCC files and processed using the R statistical language (version 3.3.1) following the procedure recommended by Nanostring. The geometrical means of the positive control probe counts were computed for each lane and a scaling factor computed for each lane being the mean of the geometrical means of all lanes divided by the geometrical mean of that particular lane. This lane specific scaling factor was then applied to all probe counts as a means to normalize for the technical variability of the platform.

The background threshold was determined for each lane using the negative controls. The threshold is the mean plus 2 standard deviations (SD) of the negative control probe counts.

The housekeeping genes *ABCF1, ALAS1, EEF1G, G6PD, GAPDH, GUSB, HPRT1, OAZ1, POLR1B, POLR2A, PPIA, RPL19, SDHA, TBP* and *TUBB* were then used to normalize for any RNA loading differences. This was performed in the same manner as the positive control probes, where the scaling factor was computed from the geometrical mean of the housekeeping genes.

The positive control and housekeeping gene normalized counts were then logarithmically transformed and used for all subsequent analysis and visualizations.

Statistical analysis

Logarithmically transformed counts of each gene were tested using ANOVAs to determine if there were any significant differences between normal, control inflammatory and HSP specimens (multiple testing correction was performed using the method of Benjamini and Hochberg). *Post-hoc t*-tests corrected for multiple testing using the method of Bonferroni were then used to determine if there were significant pairwise differences between the groups. All statistical analyses were done using the R statistical language and Excel.

Ingenuity pathway analysis

The full list of 575 genes with expression levels above background were analysed using the Ingenuity Pathway Analysis (IPA) software (March 2017 release) (Qiagen, Redwood City, CA, USA). The settings for the core analysis were as follows: user dataset; endogenous chemicals not included, direct and indirect relationships; molecules per network: 70; networks per analysis: 25. The false discovery rate or threshold was set at 0.05 to select for differentially expressed genes (DEGs) for testing. The settings were selected to compensate for the use of a targeted Nanostring panel, which has been enriched for immunological genes.