BP180-specific IgG is associated with skin adverse events, therapy response and overall survival in non-small cell lung cancer patients treated with checkpoint inhibitors

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Abstract: BACKGROUND: Anti-PD1/PD-L1 therapy frequently entails immune-related adverse events (irAEs) and biomarkers to predict irAEs are lacking. While checkpoint inhibitors have been found to re-invigorate T-cells, the relevance of autoantibodies remains elusive. OBJECTIVE: Our aim was to explore whether IgG autoantibodies directed against co-expressed antigens by tumor tissue and healthy skin correlate with skin irAEs and therapy outcome. METHODS: We measured skin-specific IgG via ELISA in non-small cell lung cancer (NSCLC) patients, who received anti-PD1/PD-L1 treatment between July 2015 and September 2017 at the Kantonsspital St. Gallen. Sera were sampled at baseline and during therapy after 8 weeks. RESULTS: Analysis of publicly available tumor expression data revealed that NSCLC and skin co-express BP180, BP230 and type VII collagen. Of 40 recruited patients, 16 (40%) developed a skin irAE. Only elevated anti-BP180 IgG at baseline significantly correlated with the development of skin irAEs (P=.04), therapy response (P=.01) and overall survival (P=.04). LIMITATIONS: The patients were recruited in a single tertiary care center. CONCLUSIONS: Our data suggest that the level of anti-BP180 IgG of NSCLC patients at baseline is associated with better therapy response, overall survival and a higher probability to develop skin irAEs during anti-PD1/PD-L1 treatment.

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D.Z. has received R&D grants by Euroimmun Inc., Lübeck, Germany, for development of novel diagnostic test systems for autoantibodies in autoimmune bullous diseases.

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Previous presentation

The content of this work has not been previously presented and is not under consideration elsewhere.

Abstract

Background

Anti-PD1/PD-L1 therapy frequently entails immune-related adverse events (irAEs) and biomarkers to predict irAEs are lacking. While checkpoint inhibitors have been found to re-invigorate T-cells, the relevance of autoantibodies remains elusive.

Objective

Our aim was to explore whether IgG autoantibodies directed against co-expressed antigens by tumor tissue and healthy skin correlate with skin irAEs and therapy outcome.

Methods

We measured skin-specific IgG via ELISA in non-small cell lung cancer (NSCLC) patients, who received anti-PD1/PD-L1 treatment between July 2015 and September 2017 at the Kantonsspital St. Gallen. Sera were sampled at baseline and during therapy after 8 weeks.

Results

Analysis of publicly available tumor expression data revealed that NSCLC and skin co-express BP180, BP230 and type VII collagen. Of 40 recruited patients, 16 (40%) developed a skin irAE. Only elevated anti-BP180 IgG at baseline significantly correlated with the development of skin irAEs (P=.04), therapy response (P=.01) and overall survival (P=.04).

Limitations

The patients were recruited in a single tertiary care center.

Conclusions

Our data suggest that the level of anti-BP180 IgG of NSCLC patients at baseline is associated with better therapy response, overall survival and a higher probability to develop skin irAEs during anti-PD1/PD-L1 treatment.

Capsule summary

- The role of antibodies during anti-PD1/PD-L1 therapy for cancer patients remains elusive.
- We found a significant correlation between higher IgG against BP180 antigen and more skin irAEs, better therapy response and prolonged overall survival in non-small cell lung cancer patients, suggesting that anti-BP180 IgG levels may be considered a biomarker.

Keywords: autoantibodies; immune-related adverse events; immune checkpoint inhibitors; non-small cell lung cancer; anti-PD1; skin rash

Introduction

The clinical introduction of immune checkpoint inhibitors (ICIs) has ushered in a new era in the treatment of metastatic non-small cell lung cancer (NSCLC) and has significantly prolonged the overall survival (OS) time of NSCLC patients.[1] Previous studies have demonstrated that the Programmed Cell Death Protein-1 (PD1) is important for maintaining the balance of peripheral tolerance against self-antigens.[2] Inhibition of PD1/PD-L1 receptor/ligand pair through use of the antibodies nivolumab, pembrolizumab and atezolizumab resulted in the development of various immune-related adverse events (irAEs) during therapy. They can affect all organs with various severity and are a major limitation to their use and effectiveness.[3] Severe irAEs may require therapy interruption and treatment with systemic immunosuppressants, which potentially compromises the anti-tumor response elicited by ICIs.[4] Among patients treated for NSCLC the most frequent irAEs are pruritus and skin rashes that typically present a lichenoid inflammation.[5] Additionally, reports of primary manifestations of bullous pemphigoid (BP) during ICI therapy have emerged and it has been speculated that in this context BP may develop due to a shared immune response against antigens in cancer cells and healthy skin tissue.[6-8] While ICIs are designed to disinhibit T cells, the role of autoantibodies against B-cell target antigens and their implications for ICI therapy remains largely unknown. We hypothesize that the expression of an immunogenic B-cell targeted skin antigen in cancer tissue could trigger the production of autoantibodies that may impact therapy outcome. Examining our hypothesis required three steps: first, identification of potential B-cell targets in NSCLC that are also present in healthy skin; second, assaying autoantibodies directed towards these antigens and third, correlating autoantibody levels with therapy response, OS and the development of skin irAEs.

Materials and Methods

Gene expression profiling

For estimating the expression of skin antigens in NSCLC tumor tissue, we first retrieved publicly available RNA-sequencing data of 1.145 NSCLC tissue samples from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov). We then compared the expressed antigens to RNA expression data of healthy reference skin tissue, provided by the Genotype Tissue Expression project (GTEx, https://gtexportal.org). We then performed manual filtering via Pubmed https://www.ncbi.nlm.nih.gov/pubmed) literature searches to select only genes that have been reported to be autoantibody targets, resulting in a list of top ten B-cell targets.

Study design

To collect serum samples we initiated a prospective observational clinical study. The study was approved by the Ethics committee of Eastern Switzerland (EKOS 16/079) and conducted in accordance with the Declaration of Helsinki guidelines. We included 40 patients with NSCLC, who visited the Kantonsspital St. Gallen for treatment between July 2015 and September 2017. All patients were scheduled to receive the first cycle of ICI therapy for treatment of NSCLC within two weeks. Patients, who have been previously treated with an ICI were not admitted into this study. Written consent was obtained prior to study inclusion and no compensation was issued for participation. Patients were treated with either the anti-PD1 antibodies nivolumab (3mg/kg, $N = 26$ (65%)), pembrolizumab (2mg/kg, $N = 12$ (30%)) or the anti-PD-L1 antibody atezolizumab (1200mg, $N = 2$ (5%)). Peripheral blood was obtained prior to the first therapy administration and after 8 weeks, regardless of the

ICI used. Patient staging was performed in accordance with clinical practice guidelines.[9] Therapy response was radiologically assessed in adherence to the RECIST 1.1 criteria.[10] IrAEs grade 1 were recorded by the physician in charge at each visit in compliance with the Common Toxicology Criteria for Adverse Events (5.0). Any irAE grade 2 or above was recorded and additionally assessed a physician trained in the medical subspecialty of the affected organ.

Measurement of autoantibodies

Serum IgGs directed to the candidate antigens BP180, BP230 and type VII collagen were measured by ELISA according to the manufacturer's instructions (MESACUP anti-Skin profile kit, MBL, Nagoya, Japan).[11] For a second, independent validation 34 of 39 serum samples were available. The validation was conducted using the following three ELISA kits: for the detection of IgG against BP180 Anti-BP180-NC16A-4X, for detection of IgG against BP230 Anti-BP230CF and for the detection of IgG against type VII collagen Anti-Collagen Type VII (all Euroimmun AG, Lübeck, Germany).[12-14]

Statistical analysis

R software (version 3.5.0) was used for all statistical analyses. The "survival" (version 2.43-1) and "survminer" (version 0.4.3) packages in R were used for survival analysis. The "maxstat" (version 0.7-25) package in R was implemented to identify optimal cutpoints for IgG levels using the maximally selected log-rank statistics.[15] The minimal proportion ('minprop' argument) in each group was set to 0.30. Kaplan-Meier plots were generated for OS and patients were categorized into "high" and "low" groups based on the optimal cutpoint for continuous levels of total IgG. The

association between IgG levels at baseline and OS was examined using the log-rank test. The Mann-Whitney test was used for calculating the association between IgG titers and therapy response, irAEs and skin irAEs. OS was calculated with 39 patients, since one patient was lost to follow-up. Response data was available from 36 of 40 patients.

Results

RNA sequencing data revealed that three antigens, BP180, BP230 and type VII collagen are among the top ten B-cell targeted antigens that are overexpressed in NSCLC and present in healthy skin. Coincidentally, all three antigens are known to be targets in blistering skin diseases; BP180 and BP230 in BP and type VII collagen in epidermolysis bullosa acquisita. Among them, BP180 appears to be the most commonly expressed antigen in NSCLC, as it can be detected in 46.1% ($N = 551$) of squamous-cell-carcinoma-type NSCLC and 8.9% ($N = 554$) of adenocarcinoma-type NSCLC samples (**Figure 1** and **Table 1**).

Our prospective study cohort included 40 patients (22 (55%) men and 18 (45%) women). The median age was 67 years. 25 (63%) of patients developed at least one irAE, of which 16 (40%) affected the skin: 9 (23%) showed skin rash and 7 (18%) reported pruritus without rash. All cases of skin rash and pruritus were classified as grades 1 or 2 according to the Common Terminology Criteria for Adverse Events (CTCAE).[16] 16 (40%) patients developed other, non-skin related irAEs. Those included thyroiditis ($N = 6$ (15%), pneumonitis ($N = 4$ (10%)), colitis ($N = 3$ (8%)), hepatitis ($N = 3$ (8%)), arthritis ($N = 1$ (3%)) and nephritis ($N = 1$ (3%)).

We then measured IgGs in patient sera with specificity to BP180, BP230, and type VII collagen and correlated their levels with skin irAEs, all irAEs, therapy response and OS. Anti-BP180 IgG levels significantly correlated with better response ($P = .01$, $N = 35$), prolonged OS ($P = .04$, $N = 39$) and the development of skin irAEs ($P = .04$, $N = 40$, see **Figure 2, top panel**), however not with all irAEs ($P = .09$, $N = 40$). Neither anti-BP230 IgG nor anti-type VII collagen IgG levels showed a correlation with any outcome (see **Figure 2 middle and bottom panels**). Outcomes are summarized in **Table 2**. The median titer of anti-BP180 IgG was 6.1 U/ml (range

21.1 – 1.4), of anti-BP230 IgG 4.8 U/ml (40.5 – 1.7) and of anti-type VII collagen IgG 3.0 U/ml (16.0 – 0.8). The associations with response and OS remained significant in the ELISA validation test ($P = .01$ and $P = .02$, respectively). ELISA on 24 serum samples that were taken after 8 weeks of therapy showed comparable IgG levels without notable change, regardless of any adverse event (**Supplemental table 1**).[17]

Discussion

We investigated whether autoantibodies that target antigens expressed in skin and NSCLC tissue are associated with outcomes and irAEs of ICI therapy. While the shared reactivity of T-cells against antigens co-expressed in tumor and healthy skin has been demonstrated,[18] very little is known about the influence of autoantibodies on therapy outcome. We have previously shown that melanoma-associated antibodies may serve as potential biomarkers for ICI efficacy in melanoma patients.[19] The concept that also irAEs correlate with autoantibodies in PD1- blockade or -deficiency has been reported: Okazaki et al. have demonstrated that PD-1 deficient mice develop dilated cardiomyopathy due to autoantibodies directed against cardiac troponin I.[20] Das et al. were able to show that early B-cell changes during ICI therapy are associated with increased autoimmunity.[21] Most recently, Toi et al. reported that certain pre-existing autoantibodies are associated with better ICI therapy outcome and more irAEs.[22] However, no single autoantibody was identified that could be associated with both at the same time, a better outcome and an associated organ-specific irAE. Using our 2-step approach, first identifying antigens with a shared tumor and skin expression and then measuring them with clinically validated diagnostic ELISA, led to the discovery of anti-BP180 IgG as a single marker for better therapy response, longer OS and the development of skin irAEs. Elevated serum levels of anti-BP180 IgG are a disease hallmark of BP, which is considered a blistering and not a lichenoid skin disease. However, it has been reported that elevated anti-BP180-IgG can be detected in certain forms of lichen planus (LP), for example vulvar LP and oral LP,[23, 24] and in overlap forms, such as bullous LP and LP pemphigoides.[25-27] Furthermore, BP can clinically and histologically present with a lichenoid skin rash or pruritus *sine matariae* without the presence of blisters for

years.[25-28] It is feasible that anti-BP180 IgG at low serum levels and disinhibition through anti-PD1/PD-L1 therapy could initiate a very mild presentation of BP, as opposed to the better known full blistering BP that is associated with high anti-BP180 IgG titers. Our data, therefore, do not suggest that autoantibodies against BP180 have a causative role but rather serve as markers for BP180 overexpression in NSCLC tissue. Why BP180 is so strongly associated with those outcomes remains to be elucidated. The expression of BP180 in tumor tissue has been shown to hold an important role in the maintenance of T-cell effector function against melanoma during ICI therapy.[29] It is possible that BP180 expression in tumor tissue may hold a similar role.

A limitation of our study is the restricted cohort size of 40 patients from a single center. However, identifying significant associations despite the limited size may further merit the value of our findings. Another limitation is the overall low levels of autoantibodies that can present a challenge to clinical application. We recommend validation and definition of ELISA cut-off ranges for daily practice with larger cohorts.

Conclusions

In a cohort of 40 patients with NSCLC receiving ICIs we demonstrate that the pre-existing levels of anti-BP180 IgG may serve as a predictive biomarker for better therapy response, prolonged OS and the development of skin irAEs during treatment. These results encourage further investigations into the role of autoantibodies for ICI therapy outcomes.

Abbreviations and Acronyms

BP: Bullous pemphigoid

BP180: Bullous pemphigoid antigen 180

BP230: Bullous pemphigoid antigen 230

CTCAE: Common Terminology Criteria for Adverse Events

EKOS: Ethics Commission of Eastern Switzerland (Ethikkommission Ostschweiz)

GTEx: Genotype-Tissue Expression project (https://gtexportal.org)

ICIs: Immune checkpoint inhibitors

irAE: immune-related adverse event

LP: Lichen planus

NSCLC: Non-small cell lung cancer

OS: Overall survival

PD1: Progammed cell death protein 1

PD-L1: Programmed death-ligand 1

TCGA: The Cancer Genome Atlas (https://portal.gdc.cancer.gov)

HGNC symbol	Description / Synonym	% SCC (*N* = 551)	% AC (*N* = 594)	% total (N = 1145)
LAMB3	laminin subunit beta 3	86	81.6	83.7
TGM2	(tissue) transglutaminase 2	40.8	79.3	60.8
LAMC1	laminin subunit gamma 1	56.1	45.6	50.7
DSC3	desmocollin 3	59	1	28.9
COL17A1	**BP180**	46.1	8.9	26.8
PPL	periplakin	25.6	19.4	22.4
COL7A1	**type VII collagen** alpha 1 chain	33.9	0.8	16.7
SERPINB3	serpin family B member 3	29.6	2.7	15.6
EVPL	envoplakin	10.2	12.8	11.5
DST	**BP230**	6	1.2	3.5

Table 1: Skin genes most expressed in NSCLC tissue, with BP180, BP230 and type VII collagen (all in bold) ranking among them. Abbreviations: SCC (squamous cell carcinoma subtype of non-small cell lung cancer), AC (adenocarcinoma subtype of non-small cell lung cancer).

Outcome		Anti-BP180 IgG			Anti-BP230 IgG			Anti-Coll. VII IgG		
		High	Low	*P* value	High	Low	*P* value	High	Low	*P* value
Response[a]	***N* (%)**									
Yes	19 (48)	**11 (58)**	8 (42)	**.049**	6 (32)	13 (68)	.236	**5 (26)**	14 (74)	**.047**
No	17 (43)	4 (24)	13 (77)		2 (12)	15 (88)		0 (0)	17 (100)	
Unknown	4 (10)	2 (50)	2 (50)		2 (50)	2 (50)		0 (0)	4 (100)	
irAE										
Yes	25 (63)	13 (52)	12 (48)	.187	6 (24)	19 (76)	1.00	2 (8)	23 (92)	.345
No	15 (38)	4 (27)	11 (73)		4 (27)	11 (73)		3 (20)	12 (80)	
Skin irAEs	16 (40)									
Rash	9 (23)	5 (56)	4 (44)	.456	2 (22)	7 (78)	1.00	1 (11)	8 (89)	1.00
Pruritus	7 (18)	5 (71)	2 (29)	.113	2 (29)	5 (71)	1.00	0 (0)	7 (100)	.565
Other irAEs	16 (40)									
Thyroiditis	6 (15)	3 (50)	3 (50)	1.00	3 (50)	3 (50)	.153	1 (17)	5 (83)	1.00
Pneumonitis	3 (8)	1 (33)	2 (67)	1.00	1 (33)	2 (67)	1.00	1 (33)	2 (67)	.338
Colitis	3 (8)	1 (33)	2 (67)	1.00	1 (33)	2 (67)	1.00	0 (0)	3 (100)	1.00

Hepatitis	3 (8)	1 (33)	2 (67)	1.00	0 (0)	3 (100)	.559	0 (0)	3 (100)	1.00
Arthritis	1 (3)	1 (100)	0 (0)	.425	1 (100)	0 (0)	.250	0 (0)	0 (0)	1.00
Nephritis	1 (3)	1 (100)	0 (0)	.425	0 (0)	0 (0)	1.00	0 (0)	0 (0)	1.00

Table 2: Outcomes of patients with non-small cell lung cancer receiving checkpoint inhibitor therapy with high and low autoantibody levels.

ELISA was measured with MESACUP anti-Skin profile kit, MBL, Nagoya, Japan.

Cut-off value for high/low IgG is 8.06 U/ml, which represents the cut-off value for overall survival using maximally selected log-rank statistics for anti-BP180 IgG.

[a]Response: defined by complete or partial response at 3 months in the CT scan. Stable or progressive disease after 3 months in the CT scan equals no response.

Percentages may not add up to 100% due to rounding.

Abbreviations: Anti-Coll. VII IgG (Anti-type VII collagen IgG), irAE (immune-related adverse event

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Figure legends

Figure 1. Skin antigen expression in non-small cell lung cancer.

A, Molecular tissue fingerprinting of 1.145 non-small cell lung cancer RNA-sequencing samples reveals the 10 most expressed genes of non-small cell lung cancer (NSCLC) and skin tissue (listed in **Table 1**). Red signifies overexpression, blue lower expression, color intensity correlates with level of expression (more intense = higher level). Sequencing data from NSCLCs were obtained from The Cancer Genome Project (https://portal.gdc.cancer.gov) and of skin tissue from the Genotype Tissue Expression (GTEx) project (https://gtexportal.org).

Figure 2. Anti-skin IgG and clinical outcome parameters

Top panel: Only anti-BP180 IgG correlate with overall survival (OS; Kaplan-Meier curve, $*P = .04$), therapy response ($*P = .01$, upper right box plot) and development of skin immune-related adverse events ($*P = .04$, middle box plot). Middle and bottom panel: Neither anti-BP230 IgG, nor anti-type VII collagen IgG correspond with OS, therapy response, the development of skin irAEs or all irAEs. IrAEs were calculated with $N = 40$ and OS with $N = 39$, since one patient was lost to follow-up. Therapy response was calculated with $N = 36$, as response data was not available from 4 patients. Cut-off values for high/low IgG were calculated for OS using the maximally selected log-rank statistics: Anti-BP180 IgG: 8.06 U/ml, anti-BP230 IgG: 6.87 U/ml, anti-type VII collagen IgG: 3.35 U/ml. The y-axes of the box plots indicate IgG titers. Abbreviations: irAE, immune-related adverse event; HR, hazard ratio; U/ml, units per milliliter.

Figure 1

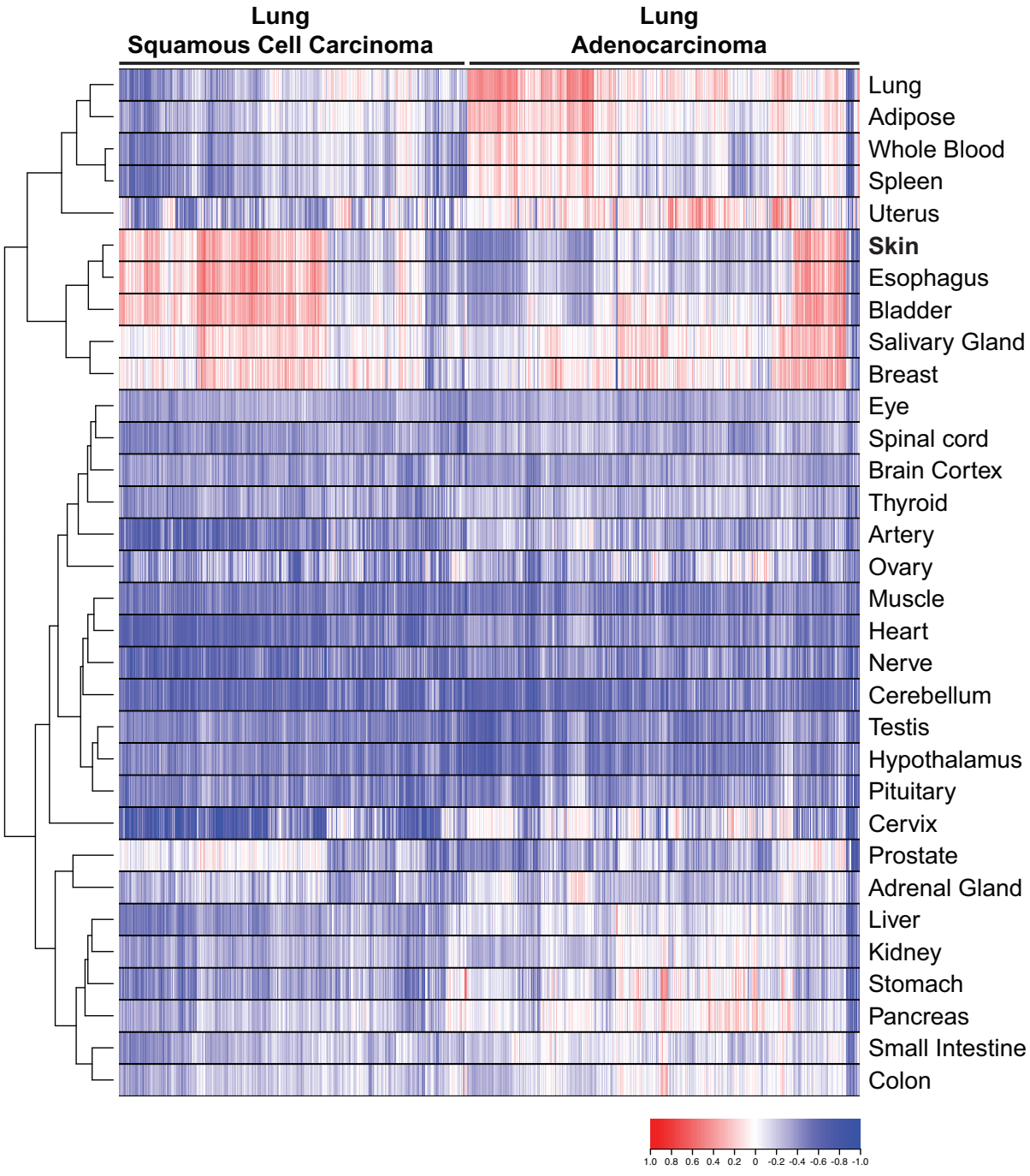
Lung
Squamous Cell Carcinoma
Lung
Adenocarcinoma
Lung
Adipose
Whole Blood
Spleen
Uterus
Skin
Esophagus
Bladder
Salivary Gland
Breast
Eye
Spinal cord
Brain Cortex
Thyroid
Artery
Ovary
Muscle
Heart
Nerve
Cerebellum
Testis
Hypothalamus
Pituitary
Cervix
Prostate
Adrenal Gland
Liver
Kidney
Stomach
Pancreas
Small Intestine
Colon
1.0 0.8 0.6 0.4 0.2 0 -0.2 -0.4 -0.6 -0.8 -1.0

Figure 2