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Utilization of Transcriptomic Profiling to Identify Molecular Markers Predicting Successful Recovery Following Endoscopic Sinus Surgery for Chronic Rhinosinusitis

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Abstract

Objectives. Successful recovery from chronic rhinosinusitis (CRS) following endoscopic sinus surgery (ESS) can be characterized by minimal presence of symptoms and absence of disease on endoscopy. However, molecular markers of surgical success remain to be characterized. These could allow for better tailoring of perioperative therapy. This study aims to identify novel molecular markers associated with surgery responsive patient.

Study Design. Prospective cohort study.

Setting. Single academic hospital center.

Method. One hundred eighteen consecutive patients with CRS at high risk of recurrence after surgery were followed prospectively following ESS in an academic medical center. Symptomatic and endoscopic outcomes were assessed at 4 months, with success rigorously defined subjectively as minimal or no symptoms (no symptom greater than 1 on an ordinal scale of 0-3) and objectively by the absence of nasal polyposis on sinus cavity endoscopy and Lund-Kennedy endoscopic edema score no greater than 1. Samples were obtained at the time of surgery and at 4-month postoperatively. Changes associated with surgery were determined by gene expression profiling using Affymetrix's Clariom S Human HT arrays.

Results. Successful ESS was characterized by a mild upregulation in Type 1 inflammation, upregulation of cell cycle progression, and epithelial barrier and proliferation-associated genes and pathways. ESS failure was associated to very high levels of Type 1 inflammation along with downregulation of epithelial barrier function and regeneration genes and pathways.

Conclusion. Successful recovery from ESS involves restoration of epithelial function and regulated activation of Type 1 inflammation. Excessively elevated Type 1 inflammation is associated with epithelial barrier dysfunction.

Keywords

barrier dysfunction, cellular senescence, chronic rhinosinusitis, endoscopic sinus surgery, epithelium, epithelial repair, gene expression, nasal polyps, Type 1 inflammation, Type 2 inflammation, wound healing, transcriptomics

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Management guidelines for chronic rhinosinusitis (CRS) recommend medical treatment initially, with surgical management reserved for patients failing appropriate medical therapy.[1] However, despite the development of considerable surgical expertise leading to minimal morbidity, the success of these procedures is not

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uniform.[2] While optimal recovery from endoscopic sinus surgery (ESS) is defined by minimal or absence of symptoms paired with absence of disease on endoscopy,[3] consistently attaining this goal is challenging. It may be difficult to obtain long-term control following surgery, and initial disease can recur rapidly in severe cases.

Contemporary concepts of the pathophysiology of CRS implicate a combination of immune dysregulation, epithelial barrier dysfunction, and microbiome dysbiosis.[4] Additionally, our understanding of CRS at the molecular level brought a change in paradigm to the field, with CRS phenotypes slowly being replaced by endotypes as means to guide therapeutic interventions.[1] Endotypes are defined by patterns of immune responses resulting from the expression of specific gene clusters and related pathways.[1,5] Most common endotypes include Type 1 (T1) associated to interferon (IFN)-γ and interleukin (IL)-12 expression, Type 2 (T2) associated to IL-4, IL-5, IL-13, and Type 3 associated to IL-17.[6,7]

This deeper understanding of CRS has continued to expand with the help of transcriptomics; the studies of whole tissue RNA sequencing. This provided a complete gene expression profiling of the given tissue and allowed for better associations between phenotypes such as eosinophilic CRS and noneosinophilic CRS to specific endotypes.[8,9] However, our understanding of how ESS modulates these endotypes is considerably more limited. Only recently, recalcitrant CRS was associated with high IL-5 and IL-13 and low IL-1β, IL-12, and IL-21.[10] This paucity of data has limited our capacity to perform effective therapeutic manipulation during the perioperative and long-term postoperative recovery period to improve outcomes.

Identifying the molecular mechanisms implicated in successful recovery from ESS could lead to improved post-ESS outcomes by delineating dysfunctional processes that may potentially be manipulated by novel therapeutic interventions. As part of larger clinical trial described elsewhere,[11,12] this study explores the mechanistic underpinnings following a successful recovery from surgery for CRS by using transcriptomics to identify implicated pathways.

Method

Clinical Trial

A transcriptomic assessment of gene expression was performed on nasal brushings previously collected during a prospective clinical trial of response to ESS performed in patients with CRS at high risk of recurrence[11] (ClinicalTrials.gov identifier: NCT02307825). This study was approved by the Ethics Review Board of the University of Montreal Health Center (Centre Hospitalier de l'Université de Montréal), file number CHUM-14.140. Briefly, a cohort of 118 patients with CRS with or without nasal polyposis at high risk of recurrence following surgery were sequentially recruited and followed prospectively. Postoperative therapy included a short course of antibiotics and a 14-day course of oral corticosteroids. Budesonide rinses were initiated at the first postoperative visit at 14 days (±3 days) and continued for trial duration. All surgeries were performed by a single surgeon and followed postoperatively by the clinical research team. Surgical success was defined using both subjective and objective measures. Symptomatic and endoscopic outcomes were assessed at 4 months, with success rigorously defined subjectively as minimal or no symptoms (no symptom greater than 1 on an ordinal scale of 0-3) and objectively by absence of nasal polyposis on sinus cavity endoscopy, with modified Lund-Kennedy endoscopic edema score no greater than 1 (on an ordinal scale of 0-2). Response to surgery was objectively defined as minimal or no symptoms with a score of ≤4 using the score. At the 4-month time point, 59 patients (50%) attained optimal outcomes, while 59 patients (50%)

Table 1. Top 25 Differentially Expressed Genes Between Surgery Unresponsive and Surgery Responsive Patients

Genes	FC[a]	*P* value[b]
Pro-inflammatory and immune-activating genes		
CXCL-8	8.1839	0.0051
TREM1	7.1956	0.0064
PLAUR	6.4887	0.0078
OSM	5.9019	0.0072
CSF1	5.5239	0.0013
CD83	5.4745	0.0017
SRGN	5.1565	0.0226
CCRL2	5.0942	0.0055
IL-1β	5.0262	0.0144
TNFAIP6	4.4112	0.0081
IL-1R2	4.0695	0.0189
Immune modulating genes		
SCGB1A1	−6.3389	0.0005
NRG4	−2.7379	0.0018
MIR99AHG	−2.4943	0.0018
Proliferation and differentiation		
BCL2A1	7.3739	0.0084
FAXDC2	−2.8715	0.0141
CD38	−2.7716	0.0336
PIK3R1	−2.5976	0.0088
CCDC103	−2.5506	0.0269
PPP1R36	−2.5111	0.0156
Repair and homeostasis		
AQP9	6.7513	0.0123
PTHLH	6.1619	0.0056
GSTA1	−2.8088	0.0164
WDR78	−2.5447	0.0303
SLC23A1	−2.4803	0.0340

Abbreviations: FC, fold change; IL, interleukin.
[a]A negative value represents downregulation in surgery unresponsive patients compared to surgery responsive patients after surgery.
[b]Represents adjusted *P* values using Fischer's exact test.

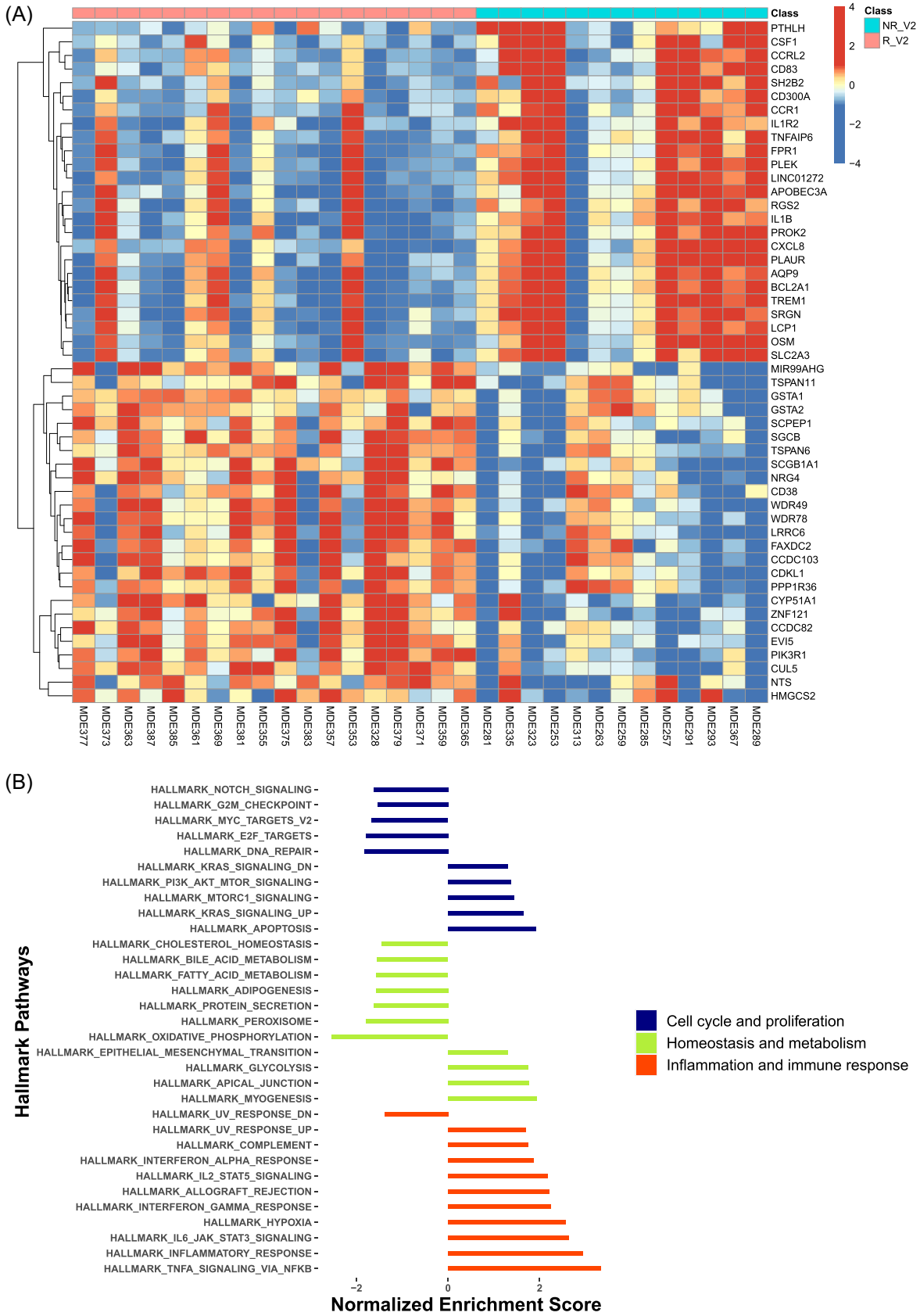

Figure 1. Postoperative surgery unresponsive versus responsive patients. (A) Heatmap of top 50 differentially expressed genes. Identified genes suggest higher expression of genes of Type I inflammation in nonresponders. (B) GSEA of selected significant pathways. Responders to surgery present a profile suggesting lower levels of Type I inflammation and increased cell proliferation, survival signaling, and DNA damage repair than surgery unresponsive patients. Presented data have an FDR < 0.05 and an absolute fold change of >1.3. FDR, False Discovery Rate; GSEA, Gene Set Enrichment Analysis.

showed persistence of disease and was deemed nonresponsive to ESS. Patients included needed at least 1 of the following criteria used to qualify them at high risk of disease recurrence: history of previous sinus surgery; sinus surgery at ≤38 years of age; absolute eosinophilia ≥500 cells/mm; total serum immunoglobulin E levels ≥150 kIU/L; sinus culture of a Gram-negative organism at any time-point; and intraoperative finding of eosinophilic mucin.[11]

Tissue samples were obtained by a mucosal brushing at the surface of the middle meatus at the time of surgery and at the 4-month time point. Total RNA was extracted from brushings using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions and stored at −80°C until microarray measurements. Gene expression was assessed using Affymetrix's Clarion S-Human-HT chips (Affymetrix, Inc.).

RNA Extraction and Gene Expression Profiling

Total RNA was extracted using RNeasy Mini Kit (Qiagen) and gene expression profiling was performed using Affymetrix's Clarion S-Human-HT Chips (Affymetrix, Inc.). However, post-hoc analysis showed an almost uniform lack of response in the patients with ASA sensitivity.

Pathway Enrichment Analysis

Gene Set Enrichment Analysis (GSEA) was performed to assess whether a known biological pathway or sets of individual genes were significantly enriched among the genes ranked by the moderated *t* test following the differential gene expression analysis. Genes associated with significant up- or downregulation of canonical pathways were considered significant when they had an adjusted $P < .05$ and an absolute fold change (FC) > 1.3. GSEA was performed using the Bioconductor's package FGSEA.[13] Tested pathways and gene sets came from the Molecular Signature Database (MsigDB, http://www.broad.mit.edu/gsea/msigdb), Hallmark (h.all.v5.0.symbols.gmt), C2 (C2.all.v6.2.symbols.gmt), and C5 (C5.all.v6.2.symbols.gmt) collections. *P* values associated to the obtained pathways following GSEA were adjusted for multiple test correction with a False Discovery Rate (FDR) cut-off significance of 0.05. In the interests of clarity, only the Hallmark data is presented.

Differential Gene Expression

Differential Gene Expression was performed using the LIMMA package from Bioconductor (R Core Team (2022), R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) by fitting a linear model for each probe and performing a moderated *t* test or *F* test on the contrast of interest.[14] The *P* values from the resulting comparison were adjusted for multiple testing according to the method of Benjamini and Hochberg,[15] with an FDR of 0.05.

Differences between surgery responsive and unresponsive patients at 4 months after ESS and prior to surgery were compared to identify gene expression changes. The mechanism of successful response and unsuccessful response to surgery was then explored by determining changes associated before and after ESS for individual patients.

Results

Comparison of gene expression results between surgery unresponsive and responsive patients at the 4-month postoperative time point showed considerable differences between the 2 groups. Among the top differentially expressed gene was MIR99AHG, a microRNA, which was downregulated in nonresponders compared to responders. Upregulation of Type 1 inflammation markers such as CXCL-8, TREMI, PLAUR, and IL-1β were

Table 2. Top 25 Differentially Expressed Genes in Surgery Responsive Patients Before and After Surgery

Genes	FC[a]	*P* value[b]
Type 2 immunity-associated genes		
CPA3	−5.1189	0.0058
TPSB2	−3.8102	0.0081
TPSAB1	−3.3376	0.0058
POSTN	−2.8896	0.0285
Immune modulating genes		
PMEPA1	3.2027	0.0001
CD163	2.7317	0.0038
CPM	2.4132	0.0127
CST1	−9.3196	0.0174
CST4	−6.5589	0.0204
ITLN1	−4.6066	0.0329
CST2	−4.5567	0.0110
Proliferation and differentiation		
KRT14	5.6383	0.0172
KRT6A	3.1081	0.0147
KRT6B	2.3736	0.0183
NTRK2	−2.7897	0.0025
EGLN3	−2.7832	0.0025
Repair and homeostasis		
MMP13	3.0168	0.0078
COL17A1	2.8472	0.0052
CDKN2A	2.7179	0.0001
MIA	2.4766	0.0254
DSG3	2.3320	0.0444
FETUB	−5.6881	0.0193
PTHLH	−4.3953	0.0290
Lipid metabolism		
ABCA1	2.8183	0.0001
CPNE8	2.6566	0.0063

Abbreviation: FC, fold change.
[a]A negative value represents downregulation after surgery compared to prior to surgery in surgery responsive patients.
[b]Represents adjusted *P* values using Fischer's exact test.

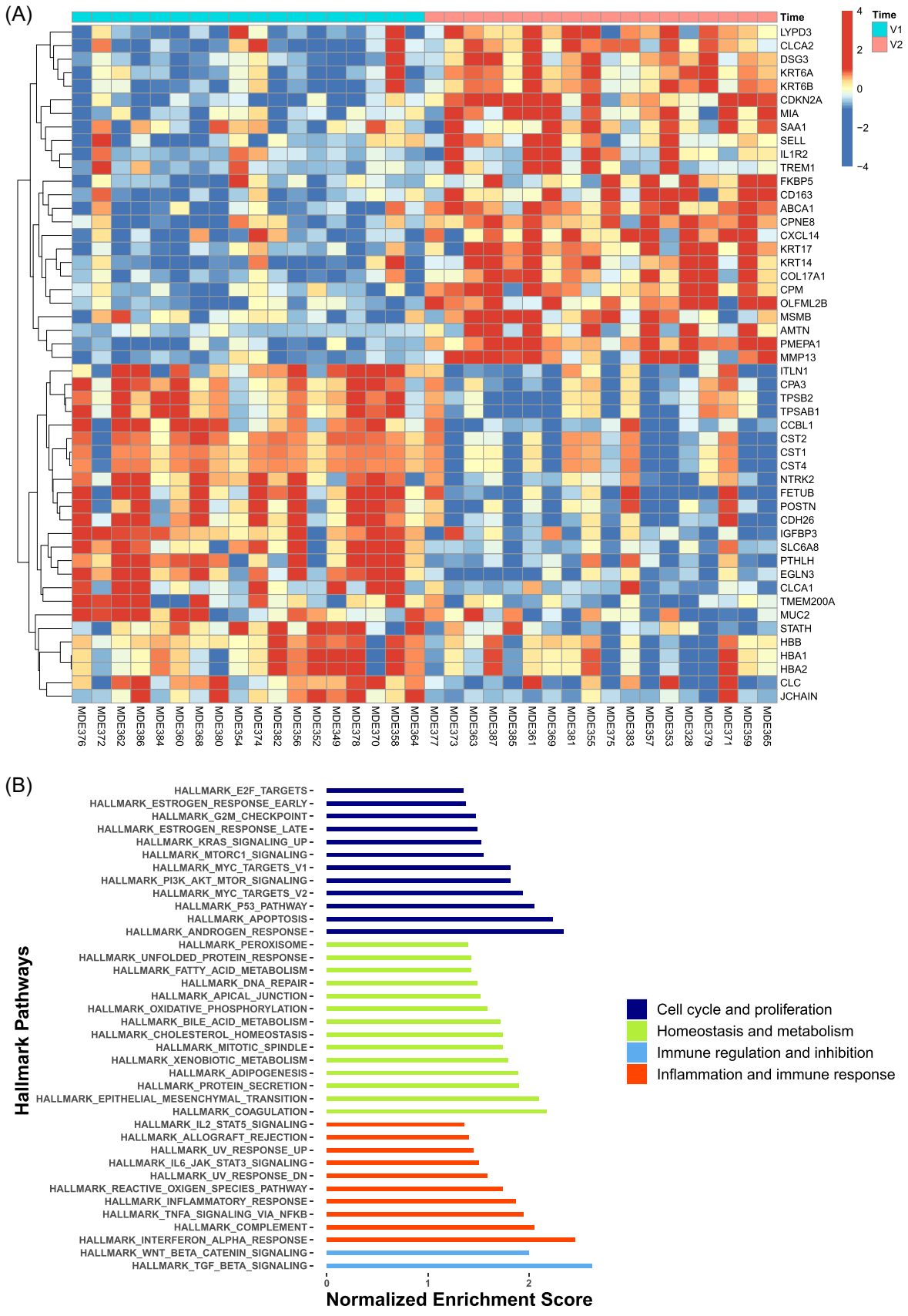

Figure 2 Continued.

observed in nonresponders (Supplemental Figure S1, available online and **Table 1**). GSEA analysis showed a significant downregulation in Hallmarks' canonical inflammatory responses pathways in the responder group corresponding to a Type 1 profile downregulation. Included in the pathways are tumor necrosis factor (TNF)-α signaling via NFκB, IL-6 JAK-STAT3 signaling, IL-2-STAT5 signaling, IFN-α, and IFN-γ responses. Epithelial-associated pathways for cell proliferation, survival signaling, and DNA damage repair involved in healing were upregulated in responders, including cholesterol homeostasis, G2M checkpoint, E2F targets, DNA repair, protein secretion, MYC targets V1 and V2, and oxidative phosphorylation (**Figure 1**).

Preoperative with postoperative gene expression comparison of surgery responsive patients was also investigated. Heatmap assessment of top 50 single gene differences suggests successful recovery from CRS is characterized by epithelial renewal, with slightly increased expression of markers for epithelial basal cells (KRT6A, KRT6B) and cytoskeletal elements (KRT17, KRT14), and a reduction in markers of Type 2 inflammation (POSTN, CLC), mast cells elements (CPA3, TPSB2, and TPSAB1), and cysteine antiproteases (CST1, CST2, CST4, FETUB) (Supplemental Figure S2, available online and **Table 2**). GSEA was characterized by marked upregulation of epithelial-associated pathways involved in healing, with identification of pathways for cell proliferation, survival signaling, and DNA damage repair, suggesting a beneficial effect on cell cycle progression and restoration of epithelial function. These pathways included cholesterol homeostasis, WNT β-catenin signaling, DNA repair, protein secretion, MYC targets V1 and V2, G2M checkpoint, and E2F targets. Moreover, pathways associated with reactive oxygen species (ROS) regulation were also upregulated, including oxidative phosphorylation, ROS, peroxisome, xenobiotic metabolism, and fatty acid metabolism pathways, suggesting a cell response that is resolving toward redox homeostasis, cell survival, and proliferation. Surprisingly, slight upregulation of Type 1 inflammatory response pathways including TNF-α signaling via NFκB, IL-6 JAK-STAT3, and IL-2-STAT5 signaling was also seen. However, the TGF-β signaling pathway involved in immune modulation by inhibiting T1 and T2 immune response while inducing Treg differentiation was the most upregulated pathway superseding the ones mentioned above (**Figure 2**).

Surgery unresponsive patients showed a slightly different gene expression pattern when comparing pre- versus postoperative. Heatmap of top 50 genes identifies upregulation of multiple markers of Type 1 inflammation (TREM1, IL-1R2, IL-1β, and TNFAIP6), accompanied by a reduction in markers of Type 2 inflammation (CLCA1, CLC) with mild downregulation of elements of immune regulators (ITLN1, SERPINB10) (Supplemental Figure S3, available online and **Table 3**). GSEA suggested a modest upregulation of Type 1 inflammation that included TNF-α signaling via nuclear factor kappa B (NFκB), IL-6 JAK-STAT3 signaling, IL-2-STAT5 signaling, IFN-α and -γ responses, and Hallmark's inflammatory response. This was coupled with the downregulation of epithelial-associated pathways for cell

Table 3. Top 25 Differentially Expressed Genes in Surgery Unresponsive Patients Before and After Surgery

Genes	FC[a]	*P* value[b]
TREM1	2.6455	3.4223
IL-1R2	2.2403	3.7512
SAA2	2.1276	5.0233
SAA1	2.0979	5.6020
IL-1β	2.0875	3.0666
OSM	2.0874	2.8201
SLC11A1	2.0279	3.7128
PDE4B	1.9797	6.0074
TNFAIP6	1.9648	3.4150
CCRL2	1.9160	2.7315
Type 2 immunity-associated genes		
CLCA1	−1.8907	−2.8133
JCHAIN	−1.7984	−3.8475
CLC	−1.4170	−2.4911
Immune modulating genes		
ITLN1	−2.0534	−3.4440
SERPINB10	−1.6168	−3.2434
SERPINB2	−1.3037	−2.7427
SERPINB11	−1.2989	−3.9404
Proliferation and differentiation		
SH2B2	2.1536	4.6724
PLEK	2.0166	3.1810
BCL2A1	1.9914	2.7540
MUC2	−1.9887	−4.0381
Repair and homeostasis		
AQP9	2.4450	3.4821
FETUB	−1.8154	−2.8317
NTS	−1.6513	−2.5096
VSIG1	−1.6227	−3.1464

Abbreviations: FC, fold change; IL, interleukin.
[a]A negative value represents downregulation after surgery compared to prior to surgery in surgery responsive patients.
[b]Represents unadjusted *P* values using Fischer's exact test.

Figure 2. Pre- versus postoperative results in surgery responsive patients. (A) Heatmap of top 50 differentially expressed genes. Identified genes suggests successful recovery from CRS is characterised by epithelial renewal and a reduction in markers of Type 2 inflammation. (B) GSEA of selected significant pathways. GSEA is characterised by marked upregulation of epithelial-associated pathways involved in healing, suggesting a beneficial effect on cell cycle progression and restoration of epithelial function. Despite favorable post-surgery evolution, a mild upregulation of Type 1 inflammatory response pathways is also seen. Presented data have an FDR < 0.05 and an absolute fold change of >1.3. FDR, False Discovery Rate; GSEA, Gene Set Enrichment Analysis.

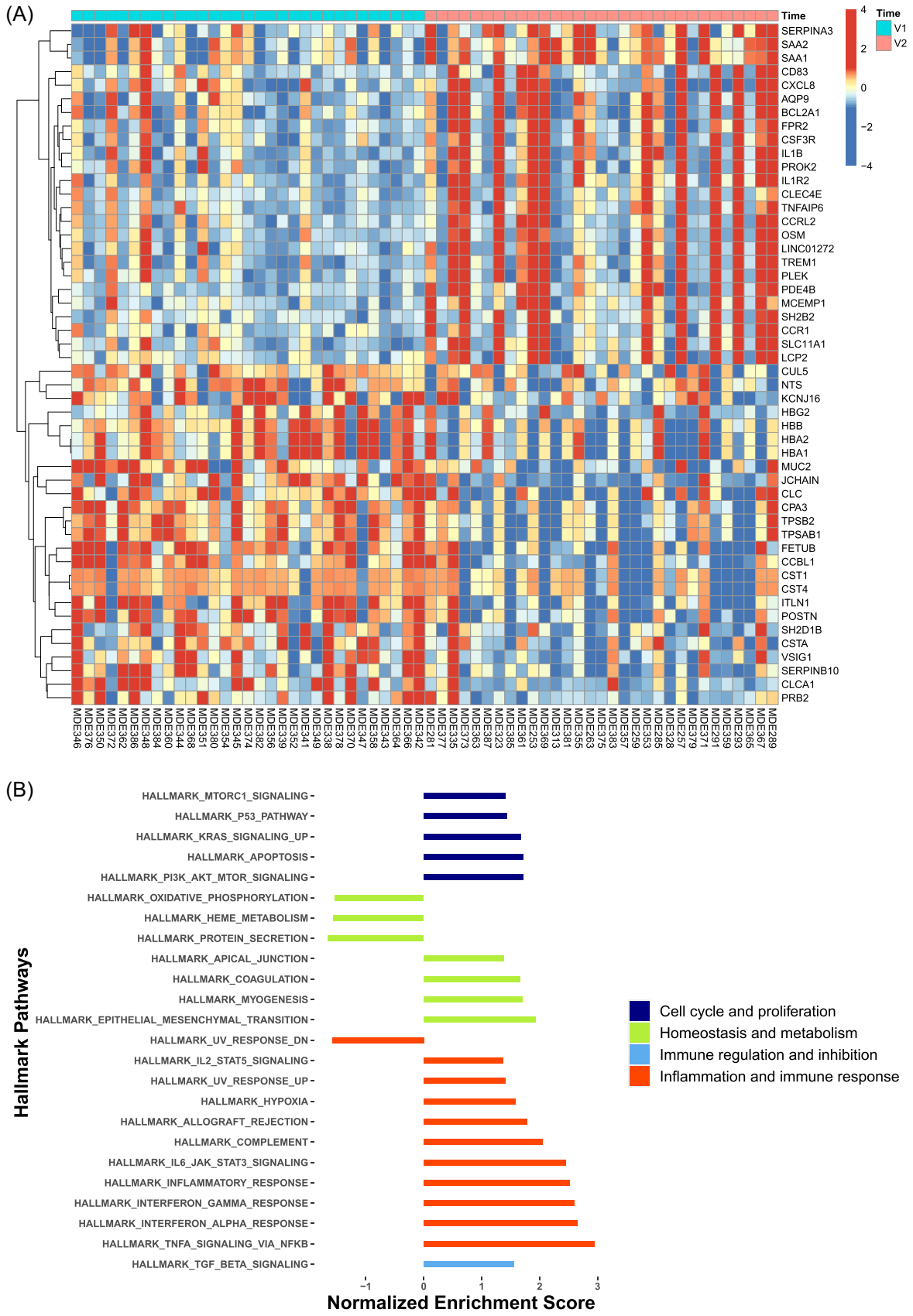

Figure 3. Pre- versus postoperative surgery unresponsive patients. (A) Heatmap of top 50 differentially expressed genes identifies upregulation of multiple markers of Type 1 inflammation by a reduction in markers of Type 2 inflammation. (B) GSEA of selected significant pathways shows modest upregulation of Type 1 inflammation, coupled with the downregulation of epithelial-associated pathways for cell proliferation, survival signaling, and DNA damage repair. Presented data have an FDR < 0.05 and an absolute fold change of >1.3. FDR, False Discovery Rate; GSEA, Gene Set Enrichment Analysis.

Table 4. Top 20 Preoperative Differentially Expressed Genes Between Surgery Responsive and Unresponsive Patients

Genes	FC[a]	*P* value[b]
Cellular metabolism and homeostasis		
VSIG1	3.1356	0.0492
LDHA	2.3736	0.0301
KLK7	2.3618	0.0492
GPRC5A	2.3153	0.0301
B3GNT6	2.3042	0.0305
HK2	2.2648	0.0492
EMP1	2.2599	0.0301
DSC2	1.8816	0.0424
SLC2A1	1.8698	0.0301
SLC16A7	1.7866	0.0492
CKAP4	1.7298	0.0391
PLEKHS1	−1.7488	0.0301
CNTRL	−1.6789	0.0467
GPR162	−1.6256	0.0301
THAP10	−1.5391	0.0401
Ciliary function		
DNALI1	−2.2715	0.0391
ARMC2	−2.2372	0.0301
CCDC60	−2.1451	0.0377
DCDC2B	−2.1208	0.0492
MRPS31	−2.0934	0.0301

Abbreviation: FC, fold change.
[a]A negative value represents downregulation in surgery unresponsive patients compared to surgery responsive patients before surgery.
[b]Represents adjusted *P* values using Fischer's exact test.

proliferation, survival signaling, and DNA damage repair including oxidative phosphorylation, UV response, and protein secretion (**Figure 3**). Preoperative comparison between surgery responsive and surgery unresponsive groups showed interesting differences. Genes involved in ciliary function and assembly were downregulated in the nonresponder along with gene involved in cell survival (VSIG1) (Supplemental Figure S4, available online and **Table 4**). Interestingly GSEA revealed that patients that eventually failed surgery had upregulated Type 1 inflammation and stress response pathways including TNF-α signaling via NFκB, IL-6 JAK-STAT3 signaling, IL-2-STAT5 signaling, IFN-α and -ɣ responses. This was accompanied by an unchanged TGF-β signaling pathway and downregulation of epithelial repair, proliferation, and survival pathways such as DNA repair, MYC targets V1, E2F targets, and oxidative phosphorylation (**Figure 4**).

Discussion

Transcriptomic results suggest that successful recovery from ESS is associated with restoration of the epithelial barrier, accompanied by a reduction in markers of Type 1 inflammation. Persistence of disease is associated with a Type-1 high inflammatory profile. While consistent with previous observations,[10] the mechanistic implications were not previously described.

In this transcriptomic assessment of mechanisms, we identify that high Type 1 inflammation is associated with apparent paralysis of the cell cycle. Our results suggest that the persistence or recurrence of disease after ESS may be considered as a failure of the epithelium to heal. This may be secondary to epithelial cell dysfunction, significantly high levels of Type 1 inflammation, or a combination of both. Impaired epithelial response to wounding has previously been demonstrated to be a characteristic feature of CRS, as previously demonstrated by in vitro scratch experiments performed by Valera et al.[16] This gene expression changes observed in the cell cycle progression may explain this epithelial fragility. The association of epithelial dysfunction in the context of high Type 1 inflammation suggests a process similar to cellular senescence.[17] Senescent cells are characterized by cell-cycle paralysis and generation of senescence-associated secreted products (SASP), which induce high T1 inflammation in neighboring cells. This is what is observed in nonresponders.

The origin of senescence-like cells in this group remains unexplained. While it is intriguing to consider the role of genetic variations as a cause of sinonasal dysfunction, they play a small role in complex diseases such as CRS.[18,19] In this context, the possibility of external agents influencing the microenvironment, such as pollution or pathogens (bacteria and/or viruses) must be included in the equation. *Staphylococcus aureus* is a ubiquitous and severe sinus pathobiont and may be a potential culprit.[20] This was described in the microbiome substudy of this trial where surgery unresponsive patients showed significantly higher levels of intranasal *S. aureus*.[12] In a link between high-T1 inflammation and *S. aureus* colonization, previous work in an elderly population manifesting constitutive Type 1 inflammation ("inflammageing") showed these patients to have the highest level of *S. aureus*.[21] Functional assessment using in vitro scratch assays confirms the link between *S. aureus* and epithelial dysfunction. In fact, *S. aureus* delayed wound healing in primary epithelial cell cultures from both normal subjects and CRS patients and is reversed by rho kinase inhibitors which promote cell differentiation and proliferation.[16]

A potential implication link with viral infection is suggested by one of the top genes differentially expressed between the two populations, miR-99AHG. miR-99AHG, also known as miR-let-7c-5p, is a noncoding microRNA, whose expression influences the downstream activation of multiple genes. miR-99AHG has a role in immune regulation, as upregulation of miR-99AHG expression inhibits the production of proinflammatory cytokines Il-1, IL-6, and TNF-α in osteoarthritis.[22] miR-99AHG expression is subject to manipulation by pathogens and is decreased following infection with respiratory syncytial virus (RSV)[23] and SARS-CoV-2 infection.[24] Restoration of function or supplementation with

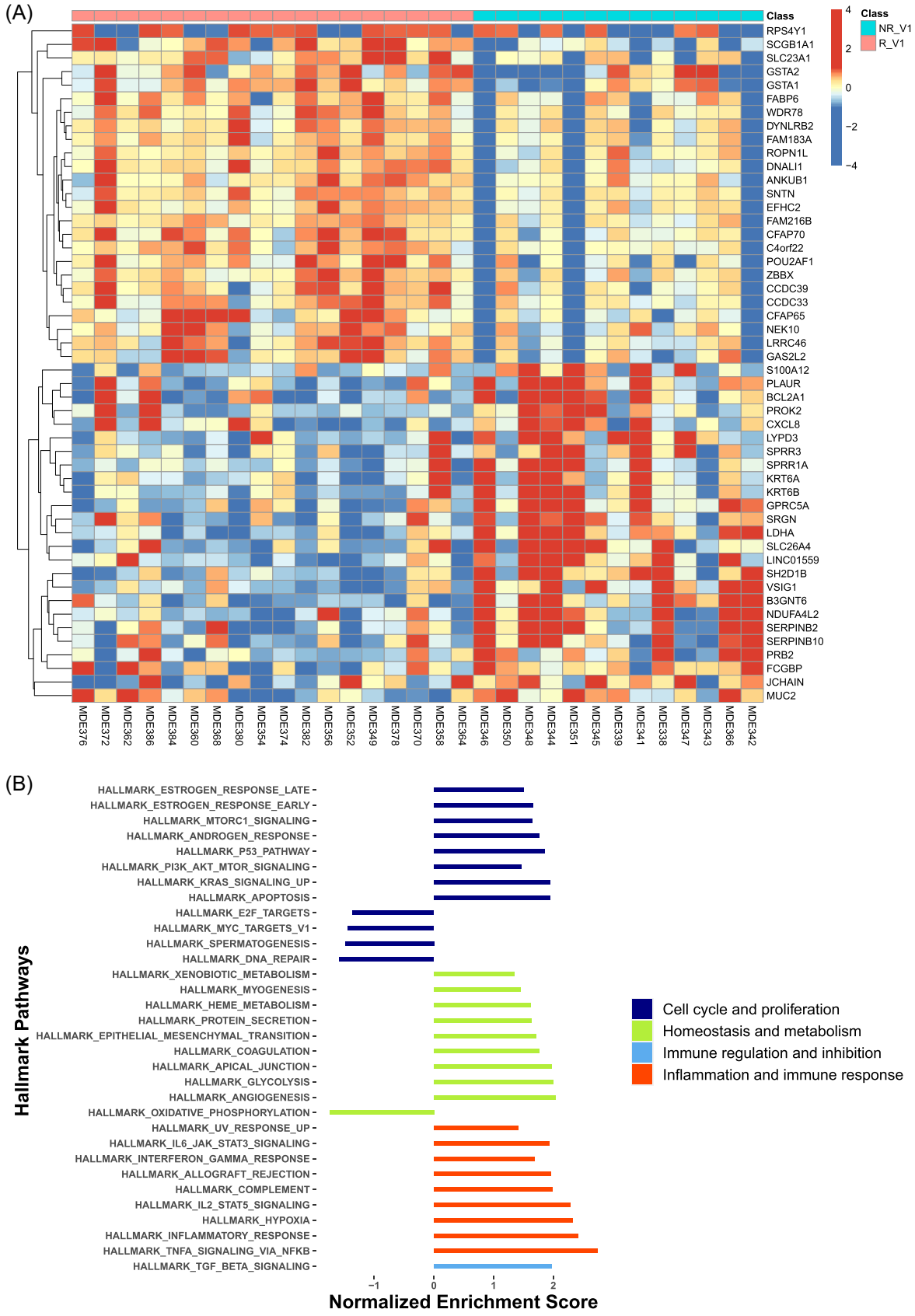

Figure 4 Continued.

miR-99AHG may have a protective effect on viral infection.[25,26] This suggests observed patterns of high Type 1 inflammation may reflect previous or persistent viral infection. Viral contribution to pathogenesis can be better targeted in future research by incorporating sequencing of the "virome."[27]

The impact on Type 2 immunity suggested by individual gene assessment was not reflected by GSEA but is nevertheless important. Reduction in Type 2 inflammation was suggested by lower expression of certain markers, mast cells, and cysteine antiproteases following surgery. However, reductions occurred in both surgery responsive and unresponsive groups. Knowing that endotypes are part of a continuum,[7] patients with predominant Type 2 inflammation may also have Type 1 inflammation that can flourish once the T2 component of the disease is treated. It is possible that surgery with the addition budesonide rinses lead to the treatment or partial treatment of T2 inflammation[28] as it is downregulated in both groups. Ultimately, pathological Type 1 inflammation may then develop postoperatively in those with a genetic, environmental, or age predisposition.

Interestingly, surgery responsive patients also demonstrated elevated Type 1 inflammation. However, this upregulation seems to have been moderated by gene involved in the TGF-β signaling pathway. This pathway tends to limit T1 and T2 immunity while promoting with Treg differentation.[29,30] This suggest T1 immunity and inflammation may lie in a delicate balance where it is needed to provide proper sinonasal epithelial healing, but in excess leads to disease. This may be better evaluated using tools capable of assessing expression patterns in individual cells such as single-cell sequencing.[31]

Finally, differences in cellular metabolism and ciliary function-associated genes were found in the preoperative period between both groups. These novel findings deserve further investigations to truly assess their role this complex disease.

Taken together, this study suggest that refractory CRS may be prevented by reducing Type 1 inflammation, improving epithelial regeneration, and repair to improve barrier function, or both simultaneously. Early administration of therapies following surgery is probably important to avoid the development of long-standing changes with possible scarring and fibrosis. These therapeutic implications are exciting but require verification in preclinical models and clinical trials prior to recommendation and diffusion.

Limitations

This study has certain limitations. In this "real-world" prospective trial, our population is heterogenous for airway comorbidities, with high frequencies of patients with asthma and aspirin-exacerbated respiratory disease, which are known to respond more poorly to surgery. Additionally, age variability may affect baseline inflammatory status. However, these difference in baseline endotypes were attenuated using postoperative budesonide rinses which uniformly lowered T2 inflammation. Additionally, preoperative GSEA demonstrated an increased T1 inflammation and cell cycle dysfunction in the eventual surgery unresponsive patients compared to the surgery responsive ones. Together, this supports the idea that excessive T1 inflammation is more likely to be refractory to surgery regardless of initial endotype or phenotype. In fact, this also may explain why some patients respond partially to biologics as once the Type 2 component of their disease is treated the T1 component remains. Nevertheless, only decreasing Type 1 inflammation without addressing the other inflammatory components may lead to failure such as Type 2 populations with AERD or asthma and nasal polyps.

More importantly, gene expression profiling represents changes in gene activity but may not reflect the level of protein product generated. Additionally, gene expression profiling does not capture the secretion of preformed mediators, and imperfectly predicts cytokine generation and secretion. These results must thus be confirmed via other methods assessing protein production and cellular function. Furthermore, additional tools will be required to determine the presence of viruses and to assess gene expression in individual cell populations.

Conclusion

In summary, response to surgery following ESS for CRS is associated with gene expression changes suggestive of cell cycle restoration with improved epithelial barrier function, innate immune signaling, and tightly regulated Type 1 inflammation, which may be interrelated phenomena. Patients with poor outcomes showed significantly higher levels of post-ESS Type 1 inflammation with concomitant epithelial dysfunction. This offers a new understanding of CRS disease and may help in the development of novel targeted therapies.

Figure 4. Preoperative surgery unresponsive versus surgery responsive patients. (A) Heatmap of top 50 differentially expressed genes. While differences are less pronounced than in the post-surgery setting, genes involved in ciliary function and assembly and genes involved cell survival are downregulated in nonresponders. (B) GSEA of selected significant pathways. Patients that eventually failed surgery showed upregulated Type 1 inflammation and stress response pathways, accompanied by downregulation of epithelial repair, proliferation and survival pathways prior to performance of surgery. Presented data have an FDR < 0.05 and an absolute fold change of >1.3. FDR, False Discovery Rate; GSEA, Gene Set Enrichment Analysis.

Author Contributions

Axel E. Renteria, patient recruitment, data analysis, literature review, preparation of manuscript; **Anastasios Maniakas**, conception of study, patient recruitment, data analysis; **Audrey Pelletier**, data analysis, literature review, preparation of manuscript; **Ali Filali-Mouhim**, Gene and pathway expression data processing and analysis; **Emmanuelle Brochiero**, data analysis and interpretation; **Fabiana C.P. Valera**, data analysis and interpretation; **Damien Adam**, data analysis and interpretation; **Leandra Endam Mfuna**, data collection and results; **Martin Desrosiers**, conception of the study, study design, data analysis, preparation of the manuscript. All co-authors contributed to the correction and revision of the manuscript

Disclosures

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Supplemental Material

Additional supporting information is available in the online version of the article.

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