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Identification of markers predictive for response to induction chemotherapy in patients with sinonasal undifferentiated carcinoma

Yoko Takahashi[a,*,1], Frederico O. Gleber-Netto[a,1], Diana Bell[b], Dianna Roberts[a], Tong-Xin Xie[a], Ahmed S. Abdelmeguid[a,2], Curtis Pickering[a], Jeffrey N. Myers[a], Ehab Y. Hanna[a]

[a] Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
[b] Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

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ABSTRACT

Objectives: Sinonasal undifferentiated carcinoma (SNUC) is a rare, highly aggressive cancer. Despite aggressive multimodal therapy, its prognosis remains poor. Because of its locally advanced nature and high propensity for distant metastasis, we frequently use induction chemotherapy before definitive therapy in patients with SNUC. However, about 30% of patients do not respond to induction chemotherapy, and lack of response is associated with a poor survival rate. Therefore, in this study, we performed gene expression analysis of SNUC samples to identify prognostic markers for induction chemotherapy response.

Materials and methods: Formalin-fixed, paraffin-embedded SNUC tumor samples from previously untreated patients harvested before induction chemotherapy were used. Gene expression was performed using an oncology gene expression panel.

Results: We identified 34 differentially expressed genes that distinguish the responders from the non-responders. Pathway analysis using these genes revealed alteration of multiple pathways between the two groups. Of these 34 genes, 24 distinguished between these two groups. Additionally, 16 gene pairs were associated with response to induction therapy.

Conclusion: We identified genes predictive of SNUC response to induction chemotherapy and pathways potentially associated with treatment outcome. This is the first report of identification of predictive biomarkers for response of SNUC to induction chemotherapy, and it may help us develop therapeutic strategies to improve the treatment outcomes of non-responders.

Introduction

Sinonasal undifferentiated carcinoma (SNUC) is a rare cancer that arises in the nasal cavity and paranasal sinuses. Initially described by Frierson et al. [1] in 1986, the latest definition of SNUC by the World Health Organization is "undifferentiated carcinoma of the sinonasal tract without glandular or squamous features and not otherwise classifiable." [2] Due to its tendency to arise near vital structures, such as the orbit, skull base, and brain, treating patient with SNUC is challenging [3–5]. Treatment usually includes aggressive multimodal therapy with radiotherapy, chemotherapy, and, in some instances, surgery [3,6–8]. Despite aggressive management of patients SNUC, their prognosis remains poor, with a median survival time of 22 months (SEER Cancer Statistics, http://seer.cancer.gov). Thus, development of new therapies is essential to improving survival of patients with SNUC.

Because of the locally advanced nature of SNUC at presentation and its high propensity for distant metastasis, we frequently use induction chemotherapy before definitive therapy in patients with SNUC [9]. A commonly used chemotherapy regimen is cisplatin (60–80 mg/m^2 on day 1) and etoposide (100–120 mg/m^2 on days 1–3) administrated intravenously every 21 days. Unfortunately, about 30% of the patients did not have responses to the treatment in a previous study, and lack of

Abbreviations: DEGs, differentially expressed genes; ECM, extracellular matrix; FFPE, formalin fixed paraffin-embedded; PI-3K, phosphoinositide 3-kinase; ROC, receiver operating characteristic; SCLC, small cell lung cancer; SNUC, sinonasal undifferentiated carcinoma

* Corresponding author at: Department of Head and Neck Surgery, Unit 123, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA.
E-mail address: ytakahas@mdanderson.org (Y. Takahashi).
[1] These authors contributed equally to this work.
[2] Address: Department of Otolaryngology Head and Neck Surgery, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

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response is associated with a poor survival rate [9]. To select optimal treatment options and help us develop new therapeutic strategies for non-responders, identifying predictive biomarkers of induction chemotherapy response in patients with SNUC is essential. However, biomarkers for prediction of induction chemotherapy response have not yet been identified.

In the study described herein, we performed comprehensive gene expression analysis of SNUC samples obtained from treatment-naïve patients to identify gene expression signatures that could predict response to induction chemotherapy.

Materials and methods

Patients and samples

For this gene expression analysis, tumor samples obtained from 13 previously untreated patients with SNUC were examined. In accordance with the Declaration of Helsinki, all patients provided written informed consent. All samples were formalin-fixed paraffin-embedded (FFPE) and were re-reviewed by a single head and neck pathologist (D.B.). Patient data were collected from an institutional database. The inclusion criterion for this study was age of 18–80 years. No patients had received treatment by the time of tissue collection, and they all subsequently received platinum-based induction chemotherapy (Supplementary Table 1). Nine of the patients had responses to induction chemotherapy, and four did not. The patient demographics and clinical characteristics are summarized in Table 1. The median follow-up time from presentation at MD Anderson to death or last contact was 31.5 months (range, 8.1–176.1 months). The study was approved by the MD Anderson Institutional Review Board.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.oraloncology.2019.07.028.

RNA expression analysis

For the gene expression analysis, a targeted RNA sequencing approach optimized for FFPE samples—the HTG EdgeSeq System (HTG Molecular Diagnostics, Tucson, AZ)—was employed. This hybridization-based system uses nuclease protection probes complementary to targeted mRNAs for gene expression measurement. In the present study, the HTG EdgeSeq Oncology Biomarker Panel, which comprises 2560 nuclease protection probes complementary to tumor biology-associated genes, was used. Sample preparation was automated using an HTG EdgeSeq Processor. Briefly, 4-μm-thick sections were obtained from the 13 FFPE SNUC samples and treated with HTG Lysis Buffer, which generates lysates containing sample RNAs. RNAs complementary to the HTG EdgeSeq Oncology Biomarker Panel probes were captured using hybridization, and unhybridized RNAs were then removed using S1 nuclease. Captured RNAs were barcoded by PCR, and sequencing adapters were added. Purified libraries were then quantified using a KAPA Library Quantification Kit (KAPA Biosystems, Wilmington, MA) and sequenced using an Ion Torrent Personal Genome Machine System (Thermo Fisher Scientific, Waltham, MA). BAM files containing gene expression data were processed using the HTG EdgeSeq host software program.

Statistical methods and pathway analysis

Differences in gene expression between responders and non-responders to induction chemotherapy were calculated using an unpaired, two-tailed *t*-test as well as the false-discovery rate *P* value (calculated using the Benjamini-Hochberg technique). Hierarchical clustering analysis was performed using Ward's minimum variance method for defining distances between clusters. Both analyses were performed using the JMP Pro 12.0.1 software program (SAS Institute, Cary, NC). Receiver operating characteristic (ROC) analysis and a two-tailed Fisher exact test were performed using the GraphPad Prism 6 software program (GraphPad Software, La Jolla, CA). Sensitivity and specificity rates and their 95% confidence intervals were calculated using the Wilson/Brown hybrid method. Pathway analysis was carried out using the KOBAS 3.0 online tool (http://kobas.cbi.pku.edu.cn), and results with corrected *P* values lower than 0.05 were considered significant [10–12].

Table 1
Clinical variables of the SNUC patients given induction chemotherapy according to treatment response.

Clinical variable		No. of patients		
		Non-responders (n = 4)	Responders (n = 9)	*P*[a]-value
Sex	Female	3	2	0.216
	Male	1	7	
Race	Asian	0	2	0.765
	Black	0	1	
	Hispanic	0	1	
	White	4	5	
Primary tumor site	Ethmoid sinus	0	6	0.074
	Frontal sinus	1	0	
	Maxillary sinus	1	1	
	Nasal cavity	2	2	
T stage	T4a	4	5	0.228
	T4b	0	4	
Lymph node metastasis	No	4	8	1.000
	Yes	0	1	
Distant metastasis	No	4	8	1.000
	Yes	0	1	
Clinical stage	IVa	4	4	0.295
	IVb	0	4	
	IVc	0	1	
Carcinogen exposure[b]	No	4	7	1.000
	Yes	0	2	
Smoking history	Former[c]	1	3	1.000
	Never	3	6	
Alcohol use	Current	1	3	0.776
	Former	2	2	
	Never	1	4	
Vital status	Alive	0	4	0.228
	Dead	4	5	
Disease-related death	No	1	2	1.000
	Yes	3	3	
Recurrence	No	0	4	0.228
	Yes	4	5	

[a] Fisher exact test.
[b] Lead or radiation.
[c] One who quite a year or more.

Predictive marker identification

To evaluate potential predictive markers in SNUC samples, the area under the curve values for all differentially expressed genes (DEGs) in responders and non-responders was calculated using ROC analysis. Genes with significant discriminatory power according to this analysis ($P < 0.05$) were considered potential predictive markers for SNUC.

After selecting these markers with predictive potential, a pairwise comparison analysis called top scoring pairs was employed [13]. Briefly, the relative expression values for all possible pairs of the selected genes were calculated. For a given gene pair (e.g., gene A and gene B), each individual sample was dichotomized according to the relative expression values (e.g., gene A > gene B or gene A < gene B). The predictive power of each gene pair was then assessed using a binary classification test.

Table 2
DEGs in responders and non-responders among treatment-naïve SNUC patients after induction chemotherapy.

Gene symbol	P	FDR q value	Mean		log2 FC
			Responders	Non-responders	
IL20	0.0024368	0.99928865	3.1	1.2	1.9
ALDH1A3	0.0041743	0.99928865	8.6	10.1	−1.5
CCL15	0.0099948	0.99928865	0.6	2.5	−1.9
SIX1	0.0137840	0.99928865	10.1	8.2	1.9
DNTT	0.0140149	0.99928865	3.0	0.6	2.3
DNAJB8	0.0142033	0.99928865	4.5	3.0	1.5
NR4A3	0.0150770	0.99928865	8.0	9.4	−1.4
FGF20	0.0151008	0.99928865	6.3	2.2	4.1
ALPL	0.0153340	0.99928865	8.3	10.3	−2.0
FBXO5	0.0166119	0.99928865	10.0	9.3	0.8
LAMB4	0.0170206	0.99928865	7.2	5.7	1.5
HSPB1	0.0204203	0.99928865	7.5	8.5	−1.0
LIF	0.0207742	0.99928865	8.7	10.6	−1.9
EFNA2	0.0215959	0.99928865	5.7	3.0	2.7
CD19	0.0255671	0.99928865	3.4	1.4	2.1
BMP2	0.0299472	0.99928865	5.7	7.1	−1.5
GNA11	0.0304962	0.99928865	10.4	10.0	0.4
MT1X	0.0309037	0.99928865	10.1	11.2	−1.2
CDC34	0.0321759	0.99928865	8.5	7.8	0.7
CCL1	0.0330950	0.99928865	3.1	5.0	−1.9
BAI1	0.0355599	0.99928865	6.3	3.9	2.4
HELLS	0.0391363	0.99928865	10.3	9.6	0.6
IL9	0.0396330	0.99928865	2.3	0.4	1.9
KRT14	0.0409939	0.99928865	8.6	10.5	−1.9
APAF1	0.0416748	0.99928865	7.7	6.8	0.9
ENDOG	0.0420415	0.99928865	8.5	9.4	−0.9
PIAS4	0.0434295	0.99928865	9.6	9.3	0.4
DLL1	0.0441168	0.99928865	9.7	7.5	2.2
HSPB7	0.0441265	0.99928865	7.1	9.0	−1.9
ITGB4	0.0452133	0.99928865	10.1	11.3	−1.2
CENPF	0.0453197	0.99928865	9.3	8.3	1.0
LATS1	0.0478415	0.99928865	10.6	10.0	0.7
LAMC2	0.0492637	0.99928865	9.0	10.1	−1.1
TNFRSF25	0.0495856	0.99928865	11.0	10.1	0.9

FDR false-discovery rate, *FC* fold change.

Results

Gene expression profiles associated with induction chemotherapy response in SNUC patients

To examine gene expression signatures in responders and non-responders among SNUC patients, we first identified DEGs between these two populations. Due to the small number of samples in both groups and large number of tested genes, we obtained no significant findings of false-discovery rate calculation. Therefore, considering only uncorrected *P* values with a cutoff less than 0.05, we determined 34 genes (Table 2) to be DEGs with distinct responses to induction chemotherapy. Box plots for the three most differentially expressed/the lowest *P* values between responders and non-responders are shown in Fig. 1A; those for the rest of the genes are shown in Supplementary Fig. 1.

Hierarchical clustering analysis using these 34 genes demonstrated clearly distinct gene expression profiles for the responders and non-responders (Fig. 1B). We confirmed the discriminant power of these 34 DEGs in distinguishing SNUC patients with different responses to the treatment via principle component analysis (Fig. 1C).

We also performed unsupervised clustering analysis to determine whether the expression pattern for the whole set of 2560 transcripts is associated with treatment response in SNUC patients. However, no clear gene expression profile correlated with treatment response in this analysis (Supplementary Fig. 2).

To further explore differences in molecular signatures between responders and non-responders, we performed pathway analysis of the 34 DEGs using KOBAS. All of the statistically significant pathways with corrected *P*-values less than 0.05 are listed in Supplementary Table 2 and summarized in Table 3. Pathways related to immune response, cell-extracellular matrix (ECM) interaction, the phosphoinositide 3-kinase (PI-3 K) cascade, the cell cycle, and apoptosis were significantly enriched by DEGs between the responders and non-responders. These findings suggested that these biologic events are key molecular features that mediate response to induction chemotherapy in SNUC patients.

Identification of predictive biomarkers for induction chemotherapy response in SNUC patients

We further searched for predictive biomarkers for induction chemotherapy response. For this, we performed ROC analysis to determine which genes had the highest predictive power. Of the 34 DEGs, 24 genes had high predictive power, with area under the curve values ranging from 1.0 to 0.861 ($P < 0.05$) (Table 4), suggesting that all 24 genes are potential predictive markers for response to induction chemotherapy in SNUC patients.

To enhance the potential clinical relevance of our findings, we used the top scoring pairs algorithm. This algorithm selects pairs of genes whose relative expression levels between two genes are consistent with two prognostic groups regardless of the gene expression assay platform [13]. First, we calculated the differences in gene expression levels for all possible gene pairs among the 24 genes with high predictive power, resulting in 276 gene pairs (Supplementary Fig. 3A). We then dichotomized all of the SNUC patients in this study according to the difference in relative expression of the genes in each pair (e.g., gene A > gene B or gene A < gene B) (Supplementary Fig. 3B). We then examined the association of this case categorization with the treatment response to evaluate the prognostic power of these gene pairs (Supplementary Fig. 3C). We found that 16 gene pairs were significantly associated with response to induction therapy (Table 5). Specifically, two gene pairs—*EFNA2-CCL1* and *CCL15-CD19*—had maximum sensitivity and specificity: lower expression of *EFNA2* than of *CCL1* and higher expression of *CCL15* than of *CD19* were highly associated with chemotherapy resistance ($P = 0.0014$ and $P = 0.0014$, respectively).

Discussion

The aim of this study was to discover molecular characteristics involved in treatment resistance and identify predictive markers to distinguish responders from non-responders to induction chemotherapy in SNUC patients. We found alteration of multiple signaling pathways between the two groups. We also discovered potential gene signatures and predictive biomarkers for induction chemotherapy response in these groups of patients.

Our first approach was to determine DEGs between responders and non-responders to identify potential predictive markers for induction chemotherapy response. The 34 DEGs we identified distinguished the responders from the non-responders in both a hierarchal cluster analysis and principle component analysis, demonstrating that this set of genes may be associated with the chance of response to induction chemotherapy in SNUC patients. On the other hand, our attempt to distinguish the responders and non-responders according to the expression pattern for the whole set of transcripts using unsupervised cluster analysis was not successful, most likely due to the small sample size.

Pathway analysis of these 34 DEGs using KOBAS demonstrated that pathways related to the immune system, cell-ECM interaction, PI-3 K signaling, the cell cycle, and apoptosis were significantly different between the responders and non-responders. Although researchers have studied the roles of PI-3 K signaling, the cell cycle, and apoptosis in cisplatin-based chemotherapy [14–17], little is known about how the immune system affects cisplatin resistance. However, in one report, the authors stated that effector $CD8^+$ T cells abrogate tumor-associated

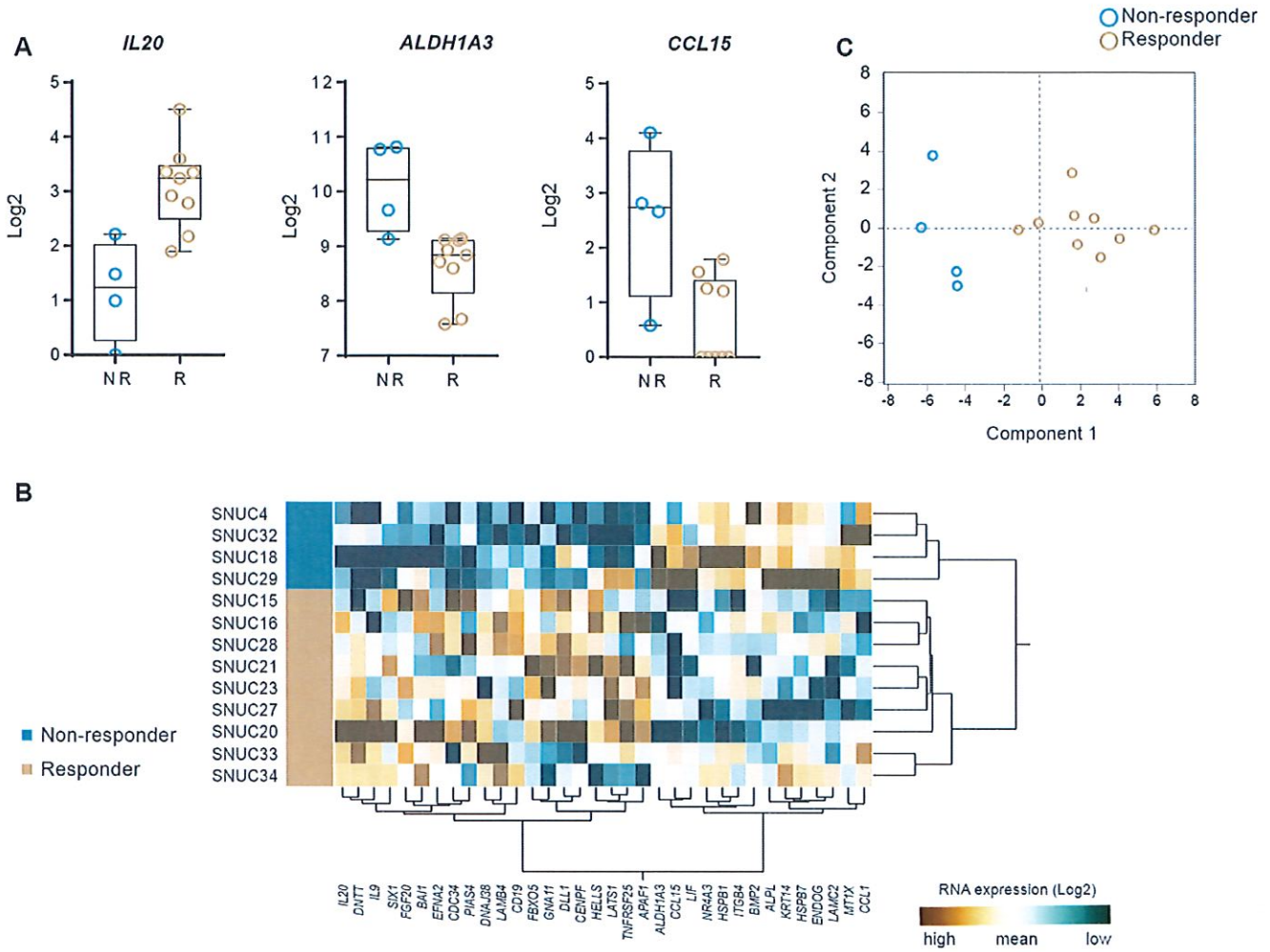

Fig. 1. DEGs between the responders and non-responders in the SNUC patients. Thirty-four DEGs were analyzed. **A** Gene expression box plots for the three genes with the lowest *P* values between responders and non-responders are listed. **B** Hierarchical cluster analysis of the 34 genes demonstrating clearly distinct gene expression profiles between the responders and non-responders. **C** Principle component analysis of the 34 genes confirming the complete distinction between the responders and non-responders.

Table 3
Pathways enriched by DEGs in responders and non-responders among SNUC patients after induction chemotherapy.

Pathway	Database	Pathway ID	*P*	Corrected *P*	Input genes
Cytokine-cytokine receptor interaction	KEGG Pathway	hsa04060	2.96E-09	1.91E-07	IL9, CCL1, CCL15, IL20, BMP2, LIF, TNFRSF25
Type I hemidesmosome assembly	Reactome	R-HSA-446107	2.06E-07	5.53E-06	ITGB4, KRT14, LAMC2
PI3K/Akt signaling pathway	KEGG Pathway	hsa04151	4.69E-07	1.09E-05	LAMB4, ITGB4, LAMC2, EFNA2, CD19, FGF20
Amoebiasis	KEGG Pathway	hsa05146	1.92E-06	3.63E-05	GNA11, HSPB1, LAMB4, LAMC2
Jak/STAT signaling pathway	KEGG Pathway	hsa04630	1.12E-05	0.000168	PIAS4, IL20, IL9, LIF
ECM-receptor interaction	KEGG Pathway	hsa04512	5.36E-05	0.000611	ITGB4, LAMB4, LAMC2
Small cell lung cancer	KEGG Pathway	hsa05222	6.15E-05	0.000679	APAF1, LAMB4, LAMC2
Cell junction organization	Reactome	R-HSA-446728	6.58E-05	0.000706	ITGB4, KRT14, LAMC2
Cytokine signaling in immune system	Reactome	R-HSA-1280215	0.000185	0.001676	TNFRSF25, IL20, IL9, FGF20, LIF
Cell-cell communication	Reactome	R-HSA-1500931	0.000216	0.001905	ITGB4, KRT14, LAMC2
Immune system	Reactome	R-HSA-168256	0.000334	0.002754	CDC34, IL9, IL20, LIF, TNFRSF25, CD19, FGF20
Laminin interactions	Reactome	R-HSA-3000157	0.000346	0.002807	ITGB4, LAMC2
Signaling by interleukins	Reactome	R-HSA-449147	0.000381	0.003060	IL20, IL9, FGF20, LIF
Integrin signaling pathway	PANTHER	P00034	0.000395	0.003141	ITGB4, LAMB4, LAMC2
Focal adhesion	KEGG Pathway	hsa04510	0.000727	0.004930	ITGB4, LAMB4, LAMC2
Nonintegrin membrane-ECM interactions	Reactome	R-HSA-3000171	0.001256	0.007337	ITGB4, LAMC2
ECM organization	Reactome	R-HSA-1474244	0.001997	0.010348	ITGB4, BMP2, LAMC2
p53 pathway	PANTHER	P00059	0.002201	0.010935	APAF1, ADGRB1
Collagen formation	Reactome	R-HSA-1474290	0.002707	0.012895	ITGB4, LAMC2
Apoptosis signaling pathway	PANTHER	P00006	0.004009	0.017198	APAF1, ENDOG
Cell cycle	Reactome	R-HSA-1640170	0.015077	0.036593	CENPF, PIAS4, FBXO5

PI3K phosphoinositide 3-kinase, *STAT* signal transducer and activator of transcription.

Table 4
DEGs between responders and non-responders identified using ROC analysis.

Gene symbol	AUC	SE	P
HSPB1	1.000	0	0.005
ALDH1A3	0.972	0.042	0.009
LAMB4	0.972	0.042	0.009
IL20	0.944	0.066	0.014
ALPL	0.917	0.085	0.021
NR4A3	0.917	0.080	0.021
FBXO5	0.917	0.080	0.021
KRT14	0.903	0.089	0.025
DNTT	0.903	0.087	0.025
CCL15	0.889	0.111	0.031
ITGB4	0.889	0.111	0.031
LAMC2	0.889	0.095	0.031
CD19	0.889	0.111	0.031
CDC34	0.889	0.094	0.031
DNAJB8	0.889	0.105	0.031
EFNA2	0.889	0.095	0.031
FGF20	0.889	0.111	0.031
SIX1	0.889	0.111	0.031
IL9	0.875	0.102	0.037
CCL1	0.861	0.106	0.045
HSPB7	0.861	0.107	0.045
CENPF	0.861	0.106	0.045
GNA11	0.861	0.106	0.045
TNFRSF25	0.861	0.132	0.045

AUC area under the curve, *SE* standard error.

resistance in different ways. First, a stiff ECM acts as physical barrier to diffusion of chemotherapeutic drugs [19–21]. Second, interaction of the ECM with cancer cells via integrins activates multiple signaling pathways. For example, Sethi et al. [22] showed that the ECM inhibited apoptosis of small cell lung cancer (SCLC) cells induced by DNA-damaging drugs via β1 integrin. Moreover, this protective effect of β1 integrin was meditated by activation of PI3-K [23]. Because overriding DNA damage-induced apoptosis in SCLC cells was completely blocked by a function-blocking anti-β1 integrin antibody [22], β1 integrin may be an attractive therapeutic target for non-responders in SNUC patients, too.

Surprisingly, the ROC analysis demonstrated that 24 of the 34 DEGs individually differentiated the responders from the non-responders. In particular, the expression of *IL20* and *FGF20* differed greatly between the two groups, suggesting that these genes are useful prognostic markers for SNUC in an actual clinical setting.

Despite our success of identifying predictive markers for induction chemotherapy response in SNUC patients, the use of single transcripts as biomarkers has many limitations, since measurement of absolute transcript expression is methodology-dependent and relies on normalization through housekeeping genes. This compromises the establishment of reliable cutoff points for sample categorization and hampers the translation of gene expression-based biomarker studies to the bedside. Therefore, we employed a simple pairwise comparison of genes (top scoring pairs analysis) to counteract these limitations. Other investigators have used this approach, which has the benefit of being independent of normalization by housekeeping genes and establishment of cutoff points for using a small number of analytes [24,25]. By using this approach, we identified 16 gene pairs with potential predictive value for induction chemotherapy response in SNUC patients.

Table 5
Gene pairs significantly associated with response of SNUC to induction chemotherapy.

Gene pair	Test result	Non-responders (*n* = 4)	Responders (*n* = 9)	P[a]	Sensitivity rate (95% CI)	Specificity rate (95% CI)
EFNA2-CCL1	EFNA2 < CCL1	4	0	0.0014	1.00 (0.51–1.00)	1.00 (0.70–1.00)
	EFNA2 > CCL1	0	9			
CCL15-CD19	CCL15 > CD19	4	0	0.0014	1.00 (0.51–1.00)	1.00 (0.70–1.00)
	CCL15 < CD19	0	9			
ALPL-FBXO5	ALPL > FBXO5	4	1	0.0070	1.00 (0.51–1.00)	0.89 (0.56–0.99)
	ALPL < FBXO5	0	8			
CCL15-DNTT	CCL15 > DNTT	4	1	0.0070	1.00 (0.51–1.00)	0.89 (0.56–0.99)
	CCL15 < DNTT	0	8			
DNAJB8-CCL1	DNAJB8 < CCL1	4	1	0.0070	1.00 (0.51–1.00)	0.89 (0.56–0.99)
	DNAJB8 > CCL1	0	8			
ALPL-CENPF	ALPL > CENPF	4	1	0.0070	1.00 (0.51–1.00)	0.89 (0.56–0.99)
	ALPL < CENPF	0	8			
CCL15-IL9	CCL15 > IL9	4	1	0.0070	1.00 (0.51–1.00)	0.89 (0.56–0.99)
	CCL15 < IL9	0	8			
IL20-CCL15	IL20 < CCL15	3	0	0.0140	0.75 (0.30–0.99)	1.00 (0.70–1.00)
	IL20 > CCL15	1	9			
ITGB4-TNFRSF25	ITGB4 > TNFRSF25	3	0	0.0140	0.75 (0.30–0.99)	1.00 (0.70–1.00)
	ITGB4 < TNFRSF25	1	9			
ALDH1A3-FBXO5	ALDH1A3 > FBXO5	3	0	0.0140	0.75 (0.30–0.99)	1.00 (0.70–1.00)
	ALDH1A3 < FBXO5	1	9			
FGF20-CCL1	FGF20 < CCL1	3	0	0.0140	0.75 (0.30–0.99)	1.00 (0.70–1.00)
	FGF20 > CCL1	1	9			
CCL15-EFNA2	CCL15 > EFNA2	3	0	0.0140	0.75 (0.30–0.99)	1.00 (0.70–1.00)
	CCL15 < EFNA2	1	9			
HSPB1-CDC34	HSPB1 > CDC34	4	2	0.0210	1.00 (0.51–1.00)	0.78 (0.45–0.96)
	HSPB1 < CDC34	0	7			
FBXO5-KRT14	FBXO5 < KRT14	4	2	0.0210	1.00 (0.51–1.00)	0.78 (0.45–0.96)
	FBXO5 > KRT14	0	7			
NR4A3-CENPF	NR4A3 > CENPF	4	2	0.0210	1.00 (0.51–1.00)	0.78 (0.45–0.96)
	NR4A3 < CENPF	0	7			
ALDH1A3-CENPF	ALDH1A3 > CENPF	4	2	0.0210	1.00 (0.51–1.00)	0.78 (0.45–0.96)
	ALDH1A3 < CENPF	0	7			

CI confidential interval.
[a] Fisher exact test.

fibroblast-mediated cisplatin resistance of ovarian cancer [18]. Therefore, investigating whether $CD8^+$ T cells are more frequently found in responders than in non-responders among SNUC patients may be interesting. We have more knowledge about how the ECM is involved in chemoresistance than in the immune system. The ECM can affect drug

Although our findings require further extensive validation due to the small sample size, the inherent simplicity of using gene pairs as biomarkers will facilitate the verification and comparison of these findings at different institutions. We believe that the 16 gene pairs we identified should play an important role in selecting the optimal therapeutic approach to patients with SNUC. There is a general agreement in the literature that multimodal therapy is needed for treatment of patients with SNUC. The specific sequence of therapy however is largely debated. Our rationale for using induction chemotherapy is the relatively high risk of distant metastasis and the known value of induction chemotherapy in reducing such risk [3,4,26–28]. Another advantage to induction chemotherapy is its potential role in predicting radiation sensitivity when choosing a primary treatment modality. Recently, we reported that in SNUC patients who achieved a favorable response to induction chemotherapy, definitive chemoradiotherapy results in improved survival compared with those who undergo definitive surgery. In patients who do not achieve a favorable response to induction chemotherapy, surgery when feasible seems to provide a better chance of disease control and improved survival [9]. Therefore establishing markers of treatment response is crucial in selecting optimal therapeutic strategies for patients with SNUC.

In summary, we found that several pathways differed significantly between responders and non-responders to induction chemotherapy in SNUC patients. We also identified 34 genes that distinguished between the two groups of patients. Of these 34 genes, 24 individually identified responders and non-responders. Additionally, we identified 16 gene pairs that were significantly associated with response of patients with SNUC to induction chemotherapy. To the best of our knowledge, this is the first report of identification of predictive biomarkers for induction chemotherapy response in SNUC patients and may help us develop new therapeutic strategies to improve treatment outcomes in non-responders.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Availability of data and material

The datasets used and/analyzed during the current study are available from the corresponding author on reasonable request.

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