Transcriptome Analysis Reveals the Differential Inflammatory Effects Between Propofol and Sevoflurane During Lung Cancer Resection: a Randomized Pilot Study

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Research

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Abstract

Background: Propofol and sevoflurane are two commonly used anesthetics perioperatively. It showed that anesthetic drugs may have an effect on tumorigenesis. Previous researches mostly focused on *in vitro* experiments, the specimens collected were mostly peripheral body fluid, lacking direct evidence of the impact of anesthetic drugs on human tissues. This study aims at elucidating the effects of propofol and sevoflurane on lung cancer using next-generation sequencing through an *in vivo* experiment.

Methods: Patients were randomly assigned to a group either receiving propofol or sevoflurane during surgery. Then patients' tumor and paired normal samples were collected and sequenced by next-generation sequencing. Differentially expressed genes (DEG) were analyzed by two statistical models, then cluster analysis, PCA, GO ontology and KEGG pathways analysis were done. Candidate genes were confirmed by qRT-PCR.

Results: Two study groups demographic data were not statistically significant. Through single factor model analysis, 810 DEG in propofol group and 508 DEG in sevoflurane group were obtained. In order to better reflect the differential effects of propofol and sevoflurane, in the meanwhile reduce false positives of DEG, we used multi-factor model analysis, which resulted in 124 DEG. In PCA and cluster analysis, four groups (propofol cancer group, propofol normal group, sevoflurane cancer group, sevoflurane normal group) were separated adequately, indicating the accuracy of DEG. We chose seven significant pathways (Cellular response to interleukin-1, Chemokine-mediated signaling pathway, Chemokine signaling pathway, Cytokine-cytokine receptor interaction, Inflammatory response, Immune response, TNF signaling pathway) for downstream analysis. From qRT-PCR results, three genes (CXCR1, CXCL8 and TNFAIP3) were confirmed.

Conclusions: Through RNA-seq analysis, the effects of propofol and sevoflurane during lung cancer resection were different, and mainly differ in inflammatory related pathways which might be possibly by targeting CXCL8.

Trial registration: Trial registry number was ChiCTR1900026213 (<http://www.chictr.org.cn/showproj.aspx?proj=43733>).

Introduction

Lung cancer has been the leading cause of cancer-associated mortality in world (Zheng et al. 2015). Several treatments could be applied to lung cancer, of which surgical resection remains the most effective approach for the majority of early-stage diagnosed patients. Each year, nearly 80% of the 15 million cancer patients requires anesthesia during surgery (Sullivan et al. 2011). Propofol and sevoflurane are the most two common anesthetic agents used in surgery. However, it has been showed that anesthetic drugs may have an effect on tumorigenesis (Buschmann et al. 2020; Hiller et al. 2018; Li et al. 2020; Shapiro et al. 1981; Wu et al. 2020; Zheng et al. 2020).

Several *in vivo* studies have discovered that propofol and sevoflurane had effects in cancer. For example, when patients received propofol during lung cancer resection, their perioperative inflammatory responses and prevalence rate of intraoperative adverse reactions were significantly reduced compared with patients in sevoflurane group (Tian et al. 2017). Compared with sevoflurane anesthesia, thoracic paravertebral nerve block-propofol anesthesia can reduce the serum concentration of vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF- β) in patients during lung cancer resection (Sen et al. 2019). A recent pilot study found differentially expressed microRNA in circulating extracellular vesicles in colorectal cancer, which showed that propofol may have an inhibited effect on cell proliferation, migration and enhancing tumor cell apoptosis (Buschmann et al. 2020). One retrospective studies revealed that propofol-based total intravenous anesthesia had a better overall survival rate than volatile anesthesia (Chang et al. 2021). However, another study found that the effects of anesthetic drugs were minimal in terms of immune activity during breast cancer surgery (Oh et al. 2018). Similar conclusions were made that there were no significant differences of overall and recurrence-free survival rate between volatile anesthesia group and propofol-based anesthesia group in a nationwide retrospective cohort study (Makito et al. 2020). Overall, it was still controversial on the effects of anesthetic drugs in cancer research.

Researchers also evaluated the effects of propofol and sevoflurane on lung cancer *in vitro*. It has been demonstrated that propofol had an adverse effect on cell viability and promoted apoptosis through downregulating the miR-21-5p/MAPK10 axis in non-small cell lung cancer cell line A549 and H1299 (Wu et al. 2020). Similar results have been observed that propofol inhibits cell growth, migration, and promoted apoptosis in A549 cells (Liu and Liu 2018; Sun and Gao 2018; Xing et al. 2018; Zheng et al. 2020). However, some studies had different conclusions, which sevoflurane had an effect in apoptosis in A549 cells (Wang et al. 2018) and could suppresses metastasis of A549 by modulating hypoxia inducible factor-1 α (Liang et al. 2015).

Overall, the effects of propofol and sevoflurane on patients who underwent lung cancer surgery were still unclear. More importantly, the underlying molecular mechanism remains unexplored. In this research, we conducted an *in vivo* study, eight lung cancer patients with tumor and paired normal samples were collected, of which 4 patients under propofol and 4 patients under sevoflurane. Then we used next-generation sequencing (NGS) to get a comprehensive list of differentially expressed genes (DEG), aiming to decipher the key factors of propofol and sevoflurane on lung cancer. At the end, candidate genes were confirmed by qRT-PCR. This study sheds light on the effects of anesthesia at the whole transcriptome level, explored the possible genes and pathways involved in tumorigenesis, which provided the basis for future work.

Materials And Methods

Ethics approval and consent to participate

This prospective study was approved by the Ethics Committee of Henan Provincial People's Hospital (2019-lushen-41). Trial registry number was ChiCTR1900026213 and registered at 26 Sep 2019 (<http://www.chictr.org.cn/showproj.aspx?proj=43733>). Written informed consent and information release approvals were obtained from all patients prior to their participation in the study. The study protocol complied with the 1975 Declaration of Helsinki.

Participant's selection

This study was designed as a randomized, single-blind study, which enrolled 28 patients with lung cancer who underwent lung cancer surgery, aged 18–65 years, BMI of 18–25 kg/m² and an ASA status of I-III from Oct 2019 to May 2020.

Inclusion criteria: no history of blood disease or other metabolic disorders; no history of hormone use; no autoimmune disease; no history of radiotherapy, chemotherapy and immunotherapy.

Exclusion criteria: history of other operations; refusal to participate in the trial; drop-out from the trial; data loss; severe hypoxemia during surgery (SpO₂ below 90% over 1 min after F_iO₂ is adjusted to 100%);

Rejection criteria: Changes in surgical procedure; blood transfusion; Postoperative diagnosis confirmed not adenocarcinoma by an independent pathological; OLV duration less than 1 hour.

Patients were randomly allocated to two groups: propofol group (P group) and sevoflurane group (S group). After intravenous injection of 0.05 mg/kg midazolam, 0.3–0.5 µg/kg sufentanil, 0.2–0.3 mg/kg etomidate and 0.6–0.9 mg/kg rocuronium were administered for induction, a double-lumen bronchial tube was inserted. Mechanical ventilation was performed after induction: F_iO₂ 70%, VT 6–8 ml/kg, RR 10–14 times/min, and I:E 1:2. During one-lung ventilation, the RR was 12–16 times/min, the F_iO₂ was 70%. The other parameters remained unchanged, P_{ET}-CO₂ was maintained at 35–45 mmHg.

The anesthesia maintenance was achieved by propofol and sevoflurane in P group and S group respectively, combined with remifentanyl and intermittent intravenous infusion of cisatracurium. The BIS was maintained between 40–50, and the fluctuation ranges of the HR and MAP were maintained at no more than 20% of the baseline values. The intraoperative intravenous infusion of Ringer's solution of sodium lactate was 2–3 ml/kg/h. 1 mg/kg oxycodone was used in our study for postoperative patient pain control.

Sample collection

Once the lung specimens were harvested, the tumor tissue and the normal tissue that was at least 5 cm away from the tumor tissue were immediately collected. After washed by PBS solution and dried by filter paper, the specimens, soaked in RNA preservation solution, were put into the – 80°C refrigerator for detection. In order to make sure that it was the tumor tissue and the normal tissue harvested, the lung specimens were verified by an independent pathologist.

RNA extraction and sequencing library construction

Total RNA was extracted following the manufacturer's protocol. The libraries were sequenced on the Illumina sequencing platform (HiSeq™ 2500) and 150 bp paired-end reads were generated.

Quality control of raw reads and alignment to reference genome

Trimmomatic (Bolger et al. 2014) was used for trimming the raw reads, including removing the adapter sequences, low-quality bases and reads. After obtaining the clean reads, Hisat2 (Sirén et al. 2014) with default parameters was used to align clean reads to reference genome (version GRCH38).

Differentially expressed genes (DEG) analysis

The DESeq2 package (Love et al. 2014) was used to identify DEG.

1) Single factor model: identifying DEG between cancer and normal samples within each group (P group or S group).

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$$

where i is condition (cancer or normal), j is replicates.

2) Multi-factor model: identifying DEG across all four conditions.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \epsilon_{ijk}$$

where i is factor 1 (P group or S group), j is factor 2 (cancer or normal), k is replicates.

Other bioinformatics analysis

Cluster analysis and principal component analysis were done in R software. Gene ontology enrichment and KEGG pathway analysis were done using DAVID (<https://david.ncifcrf.gov>) (Huang et al. 2009).

Quantitative Real-time PCR (qRT-PCR) analysis

The total RNA of tissues was efficiently extracted by the EasyPure® RNA Purification Kit (Transgen Biotech, ER701-01, Beijing, China). The obtained total RNAs were used for first-strand cDNA synthesis, and their relative expression was estimated by the q-PCR method (TransStart® Top Green qPCR SuperMix, Transgen Biotech, AQ131-01, Beijing, China) on a CFX96 Touch qPCR System (Bio-Rad Laboratories, Hercules, CA, USA). β -actin was used as an internal reference gene, and the $2^{-\Delta\Delta C_t}$ method was employed to calculate the relative expression ratio of candidate genes. The specific primers were listed (Additional File 1).

Statistical analysis

Data in qRT-PCR experiments were expressed as mean $\pm$ SE of at least three independent experiments ($n \geq 3$). The P value was calculated by two-sided Student's t test. Categorical data analysis in demographics was calculated by Chi-square test.

Results

Basic information of study groups

We collected two study groups (propofol group and sevoflurane group) demographic data, including age, gender, body mass index, ASA Physical Status, smoke history, diabetes type , arterial hypertension, coronary artery disease, UICC tumor stage, duration of surgery and duration of one-lung ventilation (Table 1). All of these data were not statistically significant between groups (P value > 0.05). In order to explore the effects of propofol (P) and sevoflurane (S) during lung cancer resection, we further matched two groups and used next-generation sequencing (NGS) to reveal the molecular mechanism (Fig. 1).

Quality of NGS and mapping rate

We sequenced a total number of 16 samples, 4 patients in each anesthesia group (P or S group) with tumor and paired normal samples. However, 2 patient data (one from P group, one from S group) did not pass the quality control step, which resulted in 12 samples (sequence libraries) left. After sequencing, we obtained a total number of 170.05 GB clean reads with an average number of 14.17 GB data for each library. The sequence quality score Q30 ranged from 92.58–93.92% and the average GC content was 47.24% (Table 2). Then we mapped clean reads to human reference genome. The total mapping rate ranged from 96.87 to 97.59% and the uniquely mapped rate was 89.85–92.56% (Table 2). After mapping step, we obtained the expression level for each gene, the distributions of Fragments per Kilobase per Million (FPKM) in all 12 samples were consistent, indicating a good sequencing quality (Fig. 2).

Differentially expressed genes (DEG) identified by single factor model

We first did differential gene analysis with paired comparisons, (1) P group: compare P group cancer samples vs P group normal samples, aiming to discover the effect of propofol on lung cancer; (2) S group: compare S group cancer samples vs S group normal samples, aiming to discover the effect of sevoflurane on lung cancer. Thus, we obtained two DEG lists. The threshold was defined as $FDR \leq 0.01$ and $\log_2FC \geq \pm 2$. In total, we obtained 810 DEG in P group and 508 DEG in S group.

In order to provide a straightforward and detailed perspective on DEG expression, we used hierarchical cluster method to cluster DEG. From the heatmap, there were clearly two clusters in each group (Fig. 3A, 3B); all cancer samples were in one cluster and normal samples in another cluster, which suggested propofol and sevoflurane had effects during lung cancer resection.

We further compared these two DEG lists and found that 191 DEG were identified in both groups. Then we are wondering whether these 191 DEG can reveal the effects of the propofol and sevoflurane were

different on lung cancer or not. However, from statistical perspective, simply look at the overlap of DEG in two comparisons, is not sufficient because too many false positives will be produced when we add up data. And if we directly compare P cancer samples vs S cancer samples, it did not a good comparison due to two reasons, 1) the control group was not the same; 2) the heterogeneity of patients. Therefore, an advanced analysis was needed.

DEG identified by multi-factor model

We developed an integrated statistical model to identify DEG, which takes the information of anesthesia drugs (propofol and sevoflurane) and conditions (cancer and normal) into account (Details in Materials and Methods). The threshold was set as $FDR \leq 0.05$ and $\log_2FC \geq \pm 1$. As a result, 124 DEG were identified, which responded to not only the condition (normal or cancer), but also the effect of propofol and sevoflurane. A number of 53 DEG were down-regulated and 71 DEG were up-regulated.

After obtaining this comprehensive list of DEG, we performed a principal component analysis (PCA), a statistical procedure to transform data from high dimension into low dimension. This type of plot is useful for visualizing the overall effect of experimental conditions. In PCA figure, four groups (P cancer group, P normal group, S cancer group, S normal group) were separated adequately (Fig. 4A). More importantly, there was no overlap between groups. For example, P group and S group separated very well from principal component 1 (PC1), cancer group and normal group separated very well from principal component 2 (PC2). Overall, PC1 can explain 50% variances and PC2 can explain 23% variances.

We also performed a hierarchical cluster analysis, where the data in the same cluster displayed high similarity (Fig. 4B). From the heatmap, the P group and S group were clearly divided into two clusters; what's more, the cancer and normal conditions were also separated well in each group, indicating the accuracy of the integrated statistical model and the identified DEG. In conclusion, from these results, it suggested the effects of propofol and sevoflurane during lung cancer resection were different.

Gene ontology (GO) and KEGG pathway analysis

GO enrichment analysis and KEGG pathways analysis were performed on 124 DEG. The threshold was taken as $P\text{-value} < 0.01$. The GO analysis consists three parts: biological process, cellular component and molecular function. Terms that were significant in each part and the number of genes in each process were plotted as a bar graph (Fig. 4C). The biological process was the most interesting and informative one, the top10 significant terms in biological process were 1) positive regulation of transcription, 2) inflammatory response, 3) positive regulation of cell proliferation, 4) immune response, 5) cellular response to lipopolysaccharide, 6) calcium ion-regulated exocytosis of neurotransmitter, 7) chemokine-mediated signaling pathway, 8) cellular response to interleukin-1, 9) cellular response to tumor necrosis factor, 10) chemotaxis.

In KEGG pathway analysis, the top 10 significant pathways with their P value and the number of genes involved in each pathway were plotted in the bubble diagram (Fig. 4D), including 1) Cytokine-cytokine

receptor interaction, 2) TNF signaling pathway, 3) Malaria, 4) Salmonella infection, 5) Chemokine signaling pathway, 6) Hepatitis B, 7) Jak-STAT signaling pathway, 8) African trypanosomiasis, 9) NOD-like receptor signaling pathway, 10) Pertussis.

From GO and KEGG pathway analyses, we compared and integrated the significant processes, then chose to focus on seven pathways (Table 3) for downstream analysis. Among these pathways (Cellular response to interleukin-1, Chemokine-mediated signaling pathway, Chemokine signaling pathway, Cytokine-cytokine receptor interaction, Inflammatory response, Immune response, TNF signaling pathway), genes enrolled in these pathways were also extracted (Table 3). Several genes were involved in more than one pathway, indicating important roles in network regulation. Therefore, we chose these genes for qRT-PCR validation.

Candidate genes confirmed by qRT-PCR

We chose four genes (CCL20, CXCR1, CXCL8, TNFAIP3) for validation. In order to better reflect the differences between P normal (Pn), P cancer (Pc), S normal (Sn) and S cancer (Sc), we calculated the ratio of normal samples to cancer samples (Pn/Pc and Sn/Sc). If this ratio is bigger than 1, it means this gene was down-regulated in cancer samples. If this ratio is smaller than 1, it means this gene was up-regulated in cancer samples. From qRT-PCR, three genes (CXCR1, CXCL8 and TNFAIP3) were consistent with RNA-seq results and statistically significant between P and S (Fig. 5), whereas CCL20 showed no significance between P and S. Both CXCR1 and TNFAIP3 showed decreased expression in cancer samples (ratio > 1), however, when compared between P and S, CXCR1 depressed more under S, TNFAIP3 depressed more under P, indicating different level of responses to anesthetic drugs. CXCL8 showed increased expression in S cancer samples (ratio < 1), whereas decreased expression in P cancer samples (ratio > 1), which may suggest cancer cells responded differently to P and S during lung cancer resection.

Discussion

In this study, we aimed to elucidate the effects of propofol and sevoflurane on lung cancer using NGS. Previous researches that studied the relationship between anesthetic drugs and tumor, mostly focused on *in vitro* experiments using cell lines and *in vivo* experiments using animals. Human researches were mostly retrospective and observational studies, lacking large-scale prospective studies. Moreover, the specimens collected were mostly peripheral body fluid, lacking direct evidence of the impact of anesthetic drugs on human tumor tissues. As far as we know, this is the first *in vivo* study, using human lung normal and cancer tissues, to investigate the effects of propofol and sevoflurane on human lung cancer. We used whole transcriptome analysis to identify key factors, with a more accurate and integrated statistical model to identify DEG between four conditions simultaneously. This improved statistical model better reflected the different effects of P and S during lung cancer resection.

Inflammatory cytokines, growth factors and chemokines are rich in the tumor microenvironment, and it is demonstrated that they have dual function in facilitating tumor growth and progression (Liu et al. 2016). Among these factors, CXCL8, which belongs to the CXL family, plays a crucial role in tumorigenesis and

angiogenesis (Zhu et al. 2004). Accumulated studies have suggested that CXCL8 can recruit and activate immune cells including granulocytes and neutrophils in the pro-inflammatory environment (Pöld et al. 2004). CXCL8 mainly relies on its ligation with CXCR1 and CXCR2 while CXCL8/CXCR1 contribute to the proliferation of tumor cells (Horn et al. 2020; Liu et al. 2016). Therefore, the expression level of CXCL8 can be used as an indicator of tumor prognosis (Liu et al. 2018). In our study, the results suggested that CXCL8 showed increased expression when treated with S, while decreased expression when treated with P. This may suggest that P could reduce inflammation while S could enhance inflammation.

As a G protein-coupled receptor (GPCR), CXCR1 was considered to be the main receptor for CXCL8 involving tumorigenesis (Yang et al. 2021). The interaction between CXCR1 and CXCL8 was mediated via the N-terminal β strand of CXCR1 (Hartl et al. 2007). Recently, it was found that CXCL1 were detected on the myeloid-derived suppressor cells which were derived from tumor (Horn et al. 2020). Therefore, CXCL8 can recruit the suppressor cells to the tumor microenvironment by recognizing CXCL1. This process may inhibit the anti-tumor effect of immune cells including T cells and NK cells. Due to the significant relationship between CXCL8 and CXCL1, several preclinical studies have been conducted recently. Some clinical grade inhibitors such as Reparixin and CXCL1-antibody, were applied to block the binding of CXCL8 and CXCL1 expecting that may have enhanced antitumor effect (Liu et al. 2016). In this study, we found that CXCR1 showed decreased expression in cancer samples, indicating similar function of P and S.

Tumor necrosis factor alpha-induced protein 3 (TNFAIP3) is an important deubiquitinating enzyme that has an impact on the tumorigenesis, immune responses and inflammation (Du et al. 2019; Liao et al. 2020). The function of TNFAIP3 mainly relies on its ubiquitin degradation for intracellular protein expression (Kim et al. 2020). Recent studies suggested that TNFAIP3 may have vital role in the invasion and proliferation of lung cancer and gastric cancer (Liao et al. 2020; Wisnieski et al. 2020). Our results revealed that the expression of TNFAIP3 was decreased in cancer samples, but decreased more under P, indicating P could reduce inflammation better than S.

In this study, there are several limitations: 1) Without long-term follow-up, it is still uncertain whether P and S has any different effects in terms of survival rate of lung cancer patients after surgery; 2) The sample size was relatively small, which only three patients' NGS data in each anesthesia group were used for RNA-seq analysis. However, although the sample size was small, the sequencing quality was good and the DEG identified in the study was able to separate different groups, indicating the accuracy and reliability of DEG.

Conclusion

In summary, our study investigated the effects of P and S at whole transcriptome level using NGS during lung cancer resection, and discovered that P and S mainly influenced inflammatory related pathways. This is consistent with the previous clinical studies that P and S had different effects on prognosis of tumor in patients. However, the DEG we identified showed that P and S may have similar function in

certain genes, but also different function in other genes. Our findings reveal the complexity role of anesthesia drugs in tumorigenesis in human cancer research.

Declarations

Ethics approval and consent to participate

The study was reviewed and approved by the Ethics Committee of Henan Provincial People's Hospital, Zhengzhou, Henan, China. All patients/participants provided written informed consent.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. RNA-seq raw reads have been deposited into NCBI SRA database (PRJNA724914).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WZ designed and support the study. SC, MJ performed experiments. SW analyzed the data and wrote the manuscript with inputs from all authors. All authors read and approved the final manuscript.

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Not applicable

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Tables

Table 1
Characteristics of demographic data between propofol and sevoflurane groups

Parameter	Propofol	Sevoflurane	P value
Age (yr) *	55–70	42–71	0.49
Gender(male/female)	3/2	2/5	0.28
Body mass index (kg/m ²) *	24.8–29.7	21.5–30.1	0.36
ASA Physical Status(/)	3/2	5/2	0.68
Smoke history (yes/no)	3/2	2/5	0.28
Diabetes type (yes/no)	2/3	0/7	0.07
Arterial hypertension (yes/no)	1/4	1/6	0.79
Coronary artery disease (yes/no)	0/5	1/6	0.38
UICC tumor stage (I a/I b/ a/ b/ a/ b)	3/0/0/1/0/1	6/1/0/0/0/0	0.29
Duration of surgery (min) *	150–240	160–225	0.25
Duration of one-lung ventilation (min) *	120–210	130–195	0.21
*Data are the range (smallest to largest)			

Table 2
Sequencing quality and mapping rate in each sample

Sample	Raw Data	Clean Data	Q30	GC Content	Total Mapping Rate	Uniquely Mapped Rate
P_cancer_1	14.86G	13.21G	93.19%	47.81%	96.87%	89.85%
P_cancer_2	15.57G	13.59G	92.82%	46.71%	96.72%	90.48%
P_cancer_3	16.73G	14.95G	93.26%	47.03%	97.11%	91.29%
P_normal_1	15.75G	13.68G	92.58%	46.51%	97.17%	92.56%
P_normal_2	17.41G	15.32G	93.02%	46.35%	97.24%	92.31%
P_normal_3	15.08G	12.90G	93.56%	47.58%	97.38%	92.17%
S_cancer_1	17.60G	15.67G	93.73%	47.40%	97.45%	90.80%
S_cancer_2	15.38G	13.56G	93.80%	47.46%	97.12%	90.55%
S_cancer_3	15.26G	13.54G	93.79%	47.83%	97.06%	90.39%
S_normal_1	16.17G	14.27G	93.85%	46.58%	97.64%	92.35%
S_normal_2	16.39G	14.40G	93.91%	47.44%	97.45%	90.99%
S_normal_3	16.84G	14.96G	93.92%	48.23%	97.59%	90.70%

Table 3
Pathways used for downstream analysis

Pathway	<i>P</i> -Value	Genes
Cellular response to interleukin-1	1.17E-03	CCL20, CXCL8 , ZC3H12A
Chemokine-mediated signaling pathway	1.17E-03	CCL20, CXCR1, CXCL8
Chemokine signaling pathway	3.03E-02	CCL20, CXCR1, CXCL8
Cytokine-cytokine receptor interaction	1.77E-05	CCL20, CXCR1, CXCL8 , CSF3, IL12RB2, IL18RAP, LIF, OSM
Inflammatory response	6.43E-06	CCL20, CXCR1, CXCL8 , CYP26B1, SELE, TNFAIP3 , ZC3H12A
Immune response	1.68E-03	CCL20, CXCL8 , CSF3, FCAR
TNF signaling pathway	5.56E-04	CCL20 , FOS, LIF, SELE, TNFAIP3
Genes in bold font are used for qRT-PCR analysis		

Figures

Figure 1

Flowchart of the patient selection, matching process and next-generation sequencing (NGS) in this research.

Figure 2

Boxplot of Fragments per Kilobase per Million (FPKM) in each sample.

Figure 3

Heatmap of cluster analysis using differentially expressed genes (DEG) between normal samples (n) and cancer samples (c). A. Heatmap of DEG from Paired P group comparison. B. Heatmap of DEG from Paired S group comparison.

Figure 4

RNA-seq analysis on 124 differentially expressed genes (DEG). A. Principal component analysis (PCA). B. Heatmap of cluster analysis. C. Bar graph of GO enrichment analysis. D. Bubble gram of KEGG pathway analysis.

Figure 5

Verification of gene expression in qRT-PCR experiments. Data were expressed as mean $\pm$ SE. Significance level was indicated as: P value < 0.05 as *, P value < 0.005 as **, P value < 0.0005 as *** and non-significant as ns. Fold change was calculated from $2^{-\Delta\Delta C_t}$ values between normal (n) samples and cancer (c) samples.

Supplementary Files

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