Flura-seq for Evaluating the Effects of Different Hyperthermic Intraperitoneal Chemotherapy Regimens on the Transcriptome of Pseudomyxoma Peritonei

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1 Study Protocol

Objective: To identify the acute transcriptomic changes in Pseudomyxoma Peritonei (PMP) in response to different Hyperthermic Intraperitoneal Chemotherapy (HIPEC) Regimens (Cisplatin vs. Cisplatin + Docetaxel).

Methods: Here, we conducted a clinical trial (ClinicalTrials.gov ID: NCT06839378, Beijing Tsinghua Chang Gung Hospital Record 24753-0-02) to evaluate the acute transcriptomic response in tumors from patients receiving cisplatin or cisplatin + docetaxel HIPEC. A total of 36 patients were enrolled and randomly divided into Cisplatin group and Cisplatin + Docetaxel group, with 18 patients in each group. All patients received an intravenous loading dose of 5-FU (400 mg/m^2) before CRS + HIPEC. Post-CRS, the corresponding HIPEC regimen was administered: Cisplatin (120 mg) or Cisplatin (120 mg) + Docetaxel (120 mg) was added to 3000 mL of normal saline, heated to 43℃, and perfused at a flow rate of 400 mL/min for 60 minutes. Tumor biopsy tissues (5-10 g) from each patient were collected before and after treatment. Total RNA was extracted, and nascent RNA was isolated using Flura-seq for sequencing analysis to identify differentially expressed genes under each treatment regimen. The amount of differentially expressed genes in response to each treatment were identified.

2 Statistical Analysis

The RNA-seq raw count matrix from tumor biopsies was processed using edgeR (v3.42.0). Data underwent TMM normalization to correct for library size bias, with low-expression genes filtered (retained genes showing CPM > 1). Differential expression analysis employed a generalized linear model (GLM) with quasi-likelihood F-tests, defining significant DEGs as those meeting adj. $P < 0.05$ (Benjamini-Hochberg FDR) and log2(foldchange) > 1. For cohort-level comparisons, GraphPad Prism 8.0 was used to calculate mean and standard deviation (SD) of: (1) total DEGs per patient across bulk RNA-seq and Flura-seq cohorts, and (2) DEG counts stratified by HIPEC regimen (cisplatin vs. cisplatin+docetaxel). Inter-group differences were assessed by unpaired two-tailed Student's t-test (normality confirmed via Shapiro-Wilk, $P>0.05$), with statistical significance threshold set at $P < 0.05$.

3 Results

3.1 Clinicopathological characteristics of 36 PMP patients treated with CRS+HIPEC

The clinicopathological characteristics of 36 PMP patients were shown in Table 1. Of all patients, median age was 58 (25 - 73), including 17 (47.3%) males and 19 (52.7%) females. Histologically, 1 (2.8%) was Acellular mucin, 22 (61.1%) were low grade mucinous carcinoma peritonei, 8 (27.8%) were high grade mucinous carcinoma peritonei, 3 (8.3%) were high grade mucinous carcinoma peritonei with signet ring cells.

Table 1 Clinicopathological Characteristics of 36 PMP Patients

Characteristic	Value (%)
Gender, *n* (%)	
Male	17 (47.3)
Female	19 (52.7)
Age (years), median (range)	58 (25 - 73)
Race	
Asian	36 (100%)
Others	0
KPS, median (range)	90 (70 - 100)
Previous surgical score, *n* (%)	
0-1	27 (65.0)
2-3	9 (35.0)
Previous intravenous chemotherapy, *n* (%)	
No	11 (30.6)
Yes	25 (69.4)
Pathological grade, *n* (%)	

Acellular mucin	1 (2.8)
Low grade mucinous carcinoma peritonei	22 (61.1)
High grade mucinous carcinoma peritonei	8 (27.8)
High grade mucinous carcinoma peritonei with signet ring cells	3 (8.3)

PMP, pseudomyxoma peritonei; KPS, Karnofsky performance status score

3.2 CRS+HIPEC information

Major CRS+HIPEC parameters were listed in Table 2. Among the 36 PMP patients, the median operation duration was 706 minutes, median bleeding, plasma infusion was 200 mL and 1000 mL. There were 2 cases (5.6 %) with PCI < 20 and 22 cases (94.4 %) with PCI ≥ 20; 21 (58.3 %) cases achieved CC 0/1 and 15 (41.7 %) cases achieved CC2/3. The median organ resection was 3, and peritonectomies were 7. There were 10 (27.8 %) cases without anastomosis, 13 (36.1 %) cases with one anastomosis, 12 (33.3 %) cases with two anastomoses, and 1 (2.8 %) case with three anastomoses.

Table 2 The Parameters of CRS+HIPEC Operations in PMP Patients

Variable	**Value**
PCI score, n (%)	
< 20	2 (5.6)
≥ 20	34 (94.4)
CC score, n (%)	
0/1	21 (58.3%)
2/3	15 (41.7%)

Operation time (min), median (range)	706 (178 ~ 944)
Bleeding (mL), median (range)	200 (50 ~ 600)
RBC infusion (U), n (%)	
< 5	36 (100)
≥ 5	0 (0)
Plasma infusion (mL), median (range)	1000 (600 ~ 1600)
Ascites (mL), median (range)	500 (0 ~ 10000)
Organ resection (part), n (%)	
≤ 2	12 (33.3%)
> 2	24 (66.7%)
Peritoneum excisions (region), n (%)	
≤ 5	16 (44.4%)
> 5	20 (55.6%)
Anastomoses, n (%)	
0	10 (27.8%)
1	13 (36.1%)
2	12 (33.3%)
3	1 (2.8%)

PMP, pseudomyxoma peritonei; KPS, Karnofsky performance status score; PCI, peritoneal cancer index; CC, completeness of cytoreduction; RBC, red blood cells; NA, not available.

3.3 Transcriptomic Analysis Results

This study employed Flura-seq technology to profile dynamic changes in nascent RNA within tumor tissues before and after HIPEC treatment.

Compared to conventional bulk RNA-seq, Flura-seq demonstrated superior performance in identifying drug-responsive transcriptional signatures in patient-derived samples. It detected a significantly higher number of differentially expressed genes (DEGs) ($P < 0.0001$) and achieved ~5-fold enrichment of nascent RNA (Figure 1).

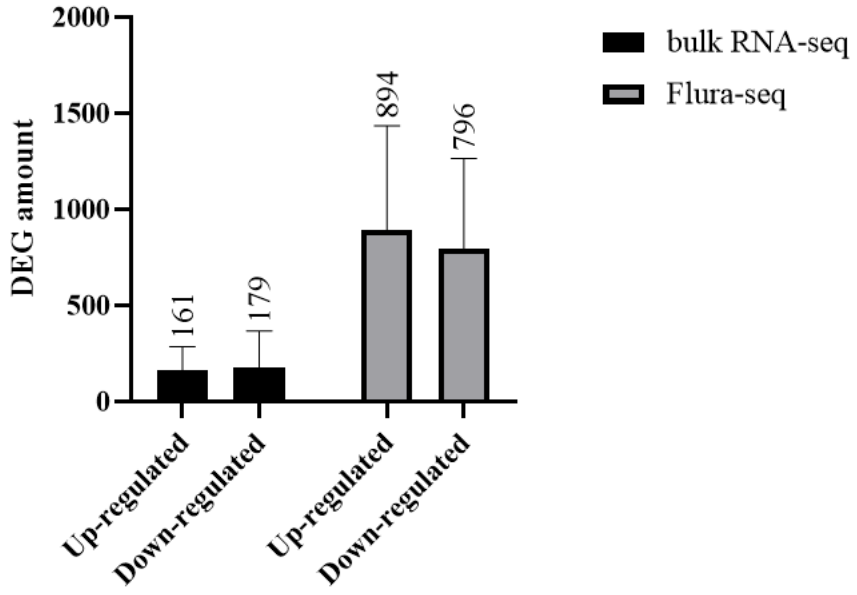

Figure 1. Comparison of DEG counts identified by Flura-seq versus bulk RNA-seq in nascent RNA sequencing of tumor tissues.

The cisplatin + docetaxel HIPEC regimen enriched ~30% more nascent RNA than cisplatin monotherapy (Figure 2).

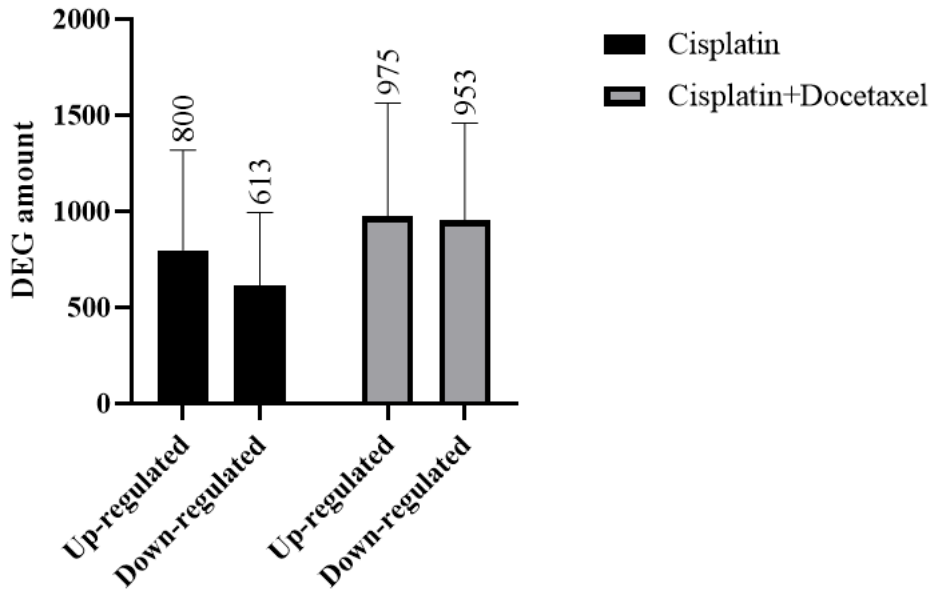

Figure 2. DEG counts in tumor tissue nascent RNA after HIPEC with cisplatin versus cisplatin + docetaxel.