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Induction Chemotherapy in Hypopharyngeal Cancer: Influence of DNA Repair Gene Polymorphisms

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Abstract. *Background/Aim: The aim was to clarify whether DNA repair gene polymorphisms can be used to predict response to cisplatin, 5-fluorouracil, and docetaxel (TPF) as induction chemotherapy (ICT) in Japanese patients with hypopharyngeal cancer (HPC). Materials and Methods: DNA repair gene polymorphisms (rs3212986, rs1799793, rs13181, and rs25487) were analyzed in 117 HPC patients and 125 control subjects by PCR-restriction fragment length polymorphism. Forty-one HPC patients who received TPF-based ICT, followed by surgery or chemoradiotherapy/radiotherapy were analyzed for ICT response, laryngeal preservation, and survival outcome. Results: ICT responders (29 cases) had significantly better overall survival than ICT non-responders (12 cases; 86.0% vs. 37.0%, respectively, p<0.01 by log-rank test) and better laryngeal preservation rates. The DNA repair gene polymorphisms were not related to ICT response. Conclusion: ICT is beneficial for chemoselection of HPC patients, but a role for DNA repair gene polymorphisms in ICT response was not confirmed.*

Significantly higher rates of laryngeal preservation have been achieved without diminishing survival outcome in patients with advanced laryngeal or hypopharyngeal cancer who received induction chemotherapy (ICT) with cisplatin and 5-fluorouracil followed by radiation therapy compared with patients who received surgery and postoperative radiation therapy (1, 2). According to previous reports (2, 3), approximately 40%-70% of patients with hypopharyngeal carcinoma (HPC) who received ICT achieved a complete or partial response with successful laryngeal preservation at the 3-year follow-up. However, the remaining cases who showed poor response needed definitive salvage surgery (*i.e.*, total pharyngo-laryngectomy). The recent standard ICT regimen of cisplatin, 5-fluorouracil, and docetaxel (TPF) (3-5) may cause severe toxic side effects. A phase II-III study that evaluated the addition of ICT to definitive chemoradiotherapy (CRT) demonstrated improved progression-free survival, locoregional control, and overall survival (OS) with induction TPF followed by cisplatin-based CRT as compared with CRT alone (6). However, this beneficial effect of ICT was not confirmed for local control or survival outcome in several phase III trials (7-9). A delay of definitive surgery due to toxic ICT may result in a poor prognosis, and it is therefore essential to identify predictive biomarkers for ICT response.

The biological cytotoxic effect of cisplatin is based on the formation of inter- and intra-strand cross-links. Alterations in the function of DNA repair genes may affect DNA repair capacity and influence the response to cisplatin-based therapy (10, 11). Excision repair cross-complementation group 1 (ERCC1) (12, 13), excision repair cross-complementation group 2/xeroderma pigmentosum group D (ERCC2/XPD), and X-ray repair cross complementing 1 (XRCC1) (14) enzymes are essential factors related to DNA repair. DNA sequencing of these enzymes has revealed the genetic polymorphisms *ERCC1* (rs3212986) (12, 13), *XPD*-312 (rs1799793), *XPD*-751 (rs13181), and *XRCC1* (rs25487) (14). These genetic polymorphisms, which exhibit ethnic variance, have been proposed as predictive biomarkers for the response to CRT in several kinds of cancers (15-19).

This study focused on whether DNA repair gene polymorphisms can be used to predict response to TPF as ICT in Japanese patients with HPC.

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Key Words: DNA repair gene, single nucleotide polymorphism, overall survival, hypopharyngeal cancer, Japanese.

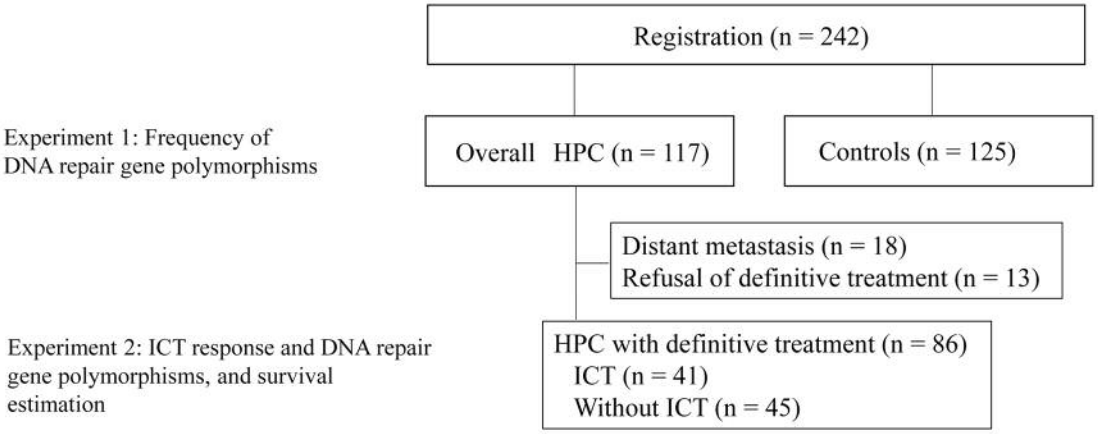

Figure 1. *Study flow diagram. HPC: Hypopharyngeal cancer; ICT: induction chemotherapy.*

Materials and Methods

Participants and study design. This study enrolled Japanese patients with HPC. All participants underwent surgery or CRT/RT with curative intent at the Department of Otorhinolaryngology, Head and Neck Surgery, University of the Ryukyus, between 2006 and 2017. DNA repair gene polymorphisms were analyzed in 117 HPC patients and 125 control subjects by DNA genotyping, as described below. A flow diagram of the study is shown in Figure 1. The controls, with no history of cancer, were treated for inflammatory disease and benign tumors, such as chronic sinusitis, chronic tonsillitis, and benign salivary tumor.

The patients' characteristics reviewed from the clinical records included age, sex, primary site, clinical stage, primary treatment, adjuvant treatment after primary treatment, pathological risk factors for recurrence, and survival information. Clinical tumor staging was performed according to the Union for International Cancer Control TNM Classification (eighth edition, 2017). Patient evaluation and the decision-making processes were conducted by head and neck surgeons and radiation oncologists before treatment was initiated. This study was approved by the institutional review board of the University of the Ryukyus and was carried out in accordance with the 1975 Declaration of Helsinki. Informed consent was obtained from all patients before enrollment.

Treatment protocols

ICT protocol. ICT was adopted for patients with T3 or T4 HPC for organ preservation (chemoselection). The basic regimen was 1 or 2 cycles of a combination of 5-fluorouracil (600 mg/m^2 on days 1-5), nedaplatin (60 mg/m^2 on day 2), and docetaxel (60 mg/m^2 on day 2). The therapeutic response was evaluated using the four categories of the Response Evaluation Criteria in Solid Tumors (RECIST; ver. 1.1): complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The initial computed tomography (CT) scan or magnetic resonance imaging scan was used as the reference image. Patients were classified according to their response to ICT: ICT responders (CR or PR) or ICT non-responders (SD or PD). The patients who achieved PR or CR to ICT underwent CRT as organ preservation treatment, and patients with SD or PD were recommended to undergo total pharyngo-laryngectomy.

CRT/RT protocol. Definitive RT (total 50.4 Gy, 1.8 Gy/day, or 50 Gy, 2 Gy/day, 5 times per week) was administered to the primary site and/or whole neck including the bilateral neck lymph nodes. The primary site and metastatic lymph nodes were subsequently treated with boost doses of a further 16.2 or 20 Gy in 9 or 10 fractions, respectively. Thus, the accumulated dose to the gross primary tumor and metastatic neck lymph nodes was 66.6 Gy or 70 Gy (once daily fraction). For CRT, the patients received platinum-based chemotherapy of either 1) 66.6 Gy RT with a combination of nedaplatin and 5-fluorouracil given twice with a 4-week interval from 2006 to 2014 or 2) 70 Gy RT with a tri-weekly infusion of 80 mg/m^2 cisplatin 3 times (20). RT alone instead of CRT was adopted in patients with stage I disease, poor renal function, or age over 75 years. If a suggestive residual lesion was observed on ultrasonography or CT at 8 weeks or on positron emission tomography-CT at 12 weeks after CRT/RT completion, salvage resection of the neck and/or primary lesion was performed.

Surgical treatment. Primary lesion removal combined with neck dissection and/or reconstructive surgery was performed in patients with advanced primary lesions (T3 or T4). As a general rule, we performed postoperative RT (60 Gy) with a tri-weekly infusion of 80 mg/m^2 cisplatin 3 times within 6 weeks after surgery if the patient had pathological high-risk factors for recurrence, namely lymph node metastasis with extracapsular extension and/or positive/close surgical margins (tumor located <5 mm from the surgical margin).

DNA extraction and single nucleotide polymorphism (SNP) genotyping. Peripheral blood samples were collected into EDTA-containing tubes and centrifuged at 2000 × *g* for 15 min. The buffy coat was stored at −80˚C until DNA extraction. Genomic DNA was extracted using a Maxwell 16 Blood purification kit (Promega, Madison, WI, USA) (21, 22). PCR-restriction fragment length polymorphism (RFLP) was performed to screen for SNPs in *ERCC1*, *XPD312*, *XPD751*, and *XRCC1* genes. The primers (*ERCC1*-8092, *XPD*-312, *XPD*-751, and *XRCC1*-399) used for PCR are listed in Table I. PCR amplification conditions were an initial denaturation step at 95˚C for 5 min, 30 cycles of denaturation at 95˚C for 30 s, annealing at 55˚C for 30 s, and extension at 72˚C for 30 s, and a final extension at 72˚C for 7 min. The genotyping procedure involved a PCR-RFLP

Table I. *Primer sequences used in this study.*

Gene	Direction	Sequence
ERCC1 (rs3212986)	Forward	GAGCCAATTCAGCCACTAGAG
	Reverse	CTTTAGTTCCTCAGTTTCCCG
XPD-312 (rs1799793)	Forward	CAGCTCATCTCTCCGCAGGATCAAAGAG
	Reverse	TAATATCGGGGCTCACCCTGCAGCACTTCCT
XPD-751 (rs13181)	Forward	CCCCCTCTCCCTTTCCTCTG
	Reverse	AACCAGGGCCAGGCAAGAC
XRCC1 (rs25487)	Forward	CCCCAAGTACAGCCAGGTC
	Reverse	TGTCCCGCTCCTCTCAGTAG

approach, performed in accordance with previously described protocols for *ERCC1*-C8092A and *XRCC1*-Arg399Gln (20).

For the *XPD*-312 polymorphism, a PCR product of 165 base pairs (bp) was obtained and digested overnight with the restriction enzyme *Sty*I (New England BioLabs, Ipswich, MA, USA). The digested PCR products were electrophoresed on 3% agarose gels, detected by staining with ethidium bromide, and visualized by illumination with UV light. The results of PCR-RFLP showed 165-bp fragments in the Asp/Asp genotype, 165-, 138-, and 27-bp fragments in the Asp/Asn genotype, or 138- and 27-bp fragments in the Asn/Asn genotype.

For the *XPD*-751 (rs13181), PCR products of 757 bp were digested with MboII at 37˚C for 1 h and electrophoresed on 2.5% agarose gels as described above. PCR-RFLP showed 98-, 131-, and 505-bp fragments in the Lys/Lys genotype, 98-, 131-, 505-, and 603-bp fragments in the Lys/Gln genotype, or 131- and 603-bp fragments in the Gln/Gln genotype.

Patient and disease characteristics according to ERCC1, XPD, or XRCC1. Patients were divided into the common homozygote (com) group and the heterozygote or polymorphic homozygote (*pol*) group according to the *ERCC1*-8092, *XPD*-312, *XPD*-751, and *XRCC1*-399 polymorphisms.

Statistical analysis. Pearson's chi-squared test was used for categorical data, and the Mann–Whitney *U*-test was used for continuous variables. Fisher's exact test was used for small samples. We compared the observed genotype frequencies with those calculated from the Hardy-Weinberg equation: $p^2 + 2pq + q^2=1$, where p is the frequency of the wild-type allele and q is $(1-p)$. Cumulative overall survival (CS) was calculated using the Kaplan–Meier method and compared within the groups using the log-rank test. The significance level was set at $p=0.05$. All analyses were carried out using JMP® 15 (SAS Institute, Inc., Cary, NC, USA).

Results

DNA repair gene polymorphism frequencies in HPC and controls. The characteristics and allele frequencies of the 117 HPC patients and 125 controls are summarized in Table II. Both groups were well matched for age and sex. *ERCC1*-8092, *XPD*-312, *XPD*-751, and *XRCC*1-399 genotype distributions in the HPC and control groups obeyed the Hardy-Weinberg equilibrium ($p=0.06$ and $p=0.93$; $p=0.08$ and $p=0.78$; $p=0.34$ and $p=0.71$; and $p=0.48$ and $p=0.35$, respectively). In the HPC and control groups, there were 9 (7.7%) and 6 (4.8%) *XPD*-312 heterozygotes or polymorphic homozygotes and 13 (11.1%) and 8 (6.4%) *XPD*-751 heterozygotes or polymorphic homozygotes, respectively. There was no significant difference in the distribution of polymorphisms between the HPC and control groups (Table II).

Correlation of ICT response, laryngeal preservation, or survival estimation with DNA repair gene polymorphisms in HPC. Of the 117 HPC patients analyzed for DNA polymorphisms, 31 were excluded because of distant metastasis or refusing definitive treatment. Finally, 86 patients with HPC were treated with curative intent (Table III). They consisted of 77 men and 9 women with a median age of 68 (range=41-88) years. The median follow-up duration was 38 months (range=8-149 months). Among these 86 patients, 48 (55.8%) had clinical stage IV disease and 43 (50.0%) underwent definitive surgery and CRT/RT as the primary treatment.

Forty-one of the 86 patients with HPC (47.7%) underwent ICT (1 cycle: 29 patients; 2 cycles: 12 patients). Of the 43 surgically treated patients, 31 (72.1%) underwent total pharyngo-laryngectomy with free jejunal flap reconstruction and 12 (27.9%) underwent transoral videolaryngoscopic resection. Pathological high-risk factors were evident in 22 (51.2%) patients (extra-nodal extension, 20 cases; positive surgical margin, 1 case; both, 1 case) and they subsequently underwent postoperative CRT. Clinical residual neck disease after CCRT was detected in 10 patients (23.3%), who subsequently underwent salvage neck dissection. Three patients developed primary recurrence after CCRT, and all were successfully salvaged with total pharyngo-laryngectomy with free jejunal flap reconstruction. Figure 2a shows the laryngeal preservation rate in ICT responders and ICT non-responders. The 3-year and 5-year laryngeal preservation rates in all 86 HPC patients were 69.5% and 65.7%, respectively. There was a significant difference ($p=0.008$) in laryngeal preservation between ICT responders

Table II. *Allele frequencies of the indicated gene variants in all HPC patients and control subjects.*

	HPC patients (n=117)	Controls (n=125)	OR (95%CI)	*p*-Value
Age (years)				
Median	68	59	N/A	<0.001
Range	38-90	16-84		
Gender				
Male	104 (81.9)	112 (89.6)	ref	
Female	13 (11.1)	13 (10.4)	1.08 (0.48-2.44)	0.86
ERCC1 (rs3212986)				
Common homozygotes CC	81 (69.2)	77 (61.6)	ref	
Heterozygotes CA	29 (24.8)	42 (33.6)	0.66 (0.37-1.16)	0.14
Polymorphic homozygotes AA	7 (6.0)	6 (4.8)	1.11 (0.36-3.45)	0.86
CA+AA	36 (30.8)	48 (38.4)	0.71 (0.42-1.22)	0.21
Alleles				
C	191 (82.9)	196 (79.4)	ref	
A	43 (18.4)	51 (20.6)	0.86 (0.55-1.35)	0.53
HWE	0.06	0.93		
XPD-312 (rs1799793)				
Common homozygotes GG	108 (92.3)	118 (94.4)	ref	
Heterozygotes GA	8 (6.8)	7 (5.6)	1.25 (0.44-3.57)	0.68
Polymorphic homozygotes AA	1 (0.9)	0	N/A	0.30
GA+AA	9 (7.7)	7 (5.6)	1.41 (0.51-3.85)	0.51
Alleles				
G	224 (95.7)	243 (97.2)	ref	
A	10 (4.3)	7 (2.8)	1.54 (0.58-4.17)	0.38
HWE	0.08	0.75		
XPD-751 (rs13181)				
Common homozygotes AA	104 (88.9)	117 (93.6)	ref	
Heterozygotes AC	12 (10.2)	8 (6.4)	1.69 (0.66-4.35)	0.27
Polymorphic homozygotes CC	1 (0.9)	0	N/A	0.29
AC+CC	13 (11.1)	8 (6.4)	1.82 (0.73-4.55)	0.19
Alleles				
A	220 (94.0)	242 (96.8)	ref	
C	14 (6.0)	8 (3.2)	1.92 (0.79-4.76)	0.14
HWE	0.34	0.71		
XRCC1 (rs25487)				
Common homozygotes GG	61 (52.1)	66 (52.8)	ref	
Heterozygotes GA	49 (41.9)	47 (37.6)	1.12 (0.66-1.92)	0.66
Polymorphic homozygotes AA	7 (6.0)	12 (9.6)	0.63 (0.23-1.69)	0.36
GA+AA	56 (47.9)	59 (47.2)	1.03 (0.62-1.69)	0.92
Alleles				
G	171 (73.1)	179 (71.6)	ref	
A	63 (26.9)	71 (28.4)	0.93 (0.63-1.39)	0.72
HWE	0.49	0.40		

HWE: Hardy-Weinberg equilibrium.

(3-year, 76.3%; 5-year, 76.3%) and ICT non-responders (3-year, 22.2%; 5-year, 22.2%).

The 5-year CS rates were 72.2% for all patients with definitive treatment (Figure 2b), 67.9% for the surgery group, and 77.1% for the CRT group. There was no significant difference in the 5-year CS rate between the surgery and CRT groups.

ICT response and cumulative survival. Of the 86 patients with HPC, 41 (47.7%) underwent ICT (1 cycle, 29 patients; 2 cycles, 12 patients). After ICT, 29 of the 41 patients (70.1%) had PR and 12 (29.9%) had SD (Table IV). None of the patients had CR to ICT or PD. Based on ICT response, 41 patients were classified into the ICT responder group (29 cases) and ICT non-responder group (12 cases) and were subjected to further analysis.

The 5-year CS rates did not differ between patients with and without ICT (71.3% *vs*. 72.9%, respectively; p=0.37; Figure 2c). The 5-year CS rate was better in ICT responders than in ICT non-responders (79.3% *vs*. 48.6%, respectively; p=0.04; Figure 2d).

Table III. *Patient, tumor, and treatment characteristics of the 86 patients with hypopharyngeal cancer.*

Number of patients	n=86
Characteristics	n (%)
Age (years)	
Median	68
Range	41-88
Gender	
Male	77 (89.5)
Female	9 (10.5)
Smoking history	
Never	6 (7.0)
Ever	35 (40.7)
Current	43 (50.0)
Unknown	2 (2.3)
Alcohol history	
Never	5 (5.8)
Ever	29 (33.7)
Current	50 (58.1)
Unknown	2 (2.3)
Primary site and subsite	
Piriform sinus	61 (70.9)
Posterior cricoid	16 (18.6)
Posterior wall	9 (10.5)
SCC	
Poorly differentiated	13 (15.1)
Moderately differentiated	52 (60.5)
Well differentiated	21 (24.4)
Clinical T classification	
T1	9 (10.5)
T2	35 (40.7)
T3	24 (27.9)
T4	18 (20.9)
Clinical N classification	
N0	37 (43.0)
N1	6 (7.0)
N2	39 (45.3)
N3	4 (4.7)
Clinical stage	
I	8 (9.3)
II	18 (20.9)
III	12 (14.0)
IV	48 (55.8)
Induction chemotherapy	
(+)	41 (47.7)
(−)	45 (52.3)
Definitive treatment	
Surgery	43 (50.0)
Radiation	43 (50.0)
Post-treatment high-risk factor	
Surgery	
Pathological high-risk factor	22/43 (51.2)
CRT/RT	
Clinical residual neck disease	10/43 (23.3)
Gene variant	
Common homozygote, heterozygote, polymorphic homozygote	
ERCC1 (rs3212986)	
C/C, C/A, A/A	62 (72.1), 19 (22.1), 5 (5.8)
XPD-312 (rs1799793)	
Asp/Asp, Asp/Asn, Asn/Asn	80 (93.0), 6 (7.0), 0 (0)
XPD-751 (rs13181)	
Lys/Lys, Lys/Gln, Gln/Gln	77 (89.5), 8 (9.3), 1 (1.2)
XRCC1 (rs25487)	
Arg/Arg, Arg/Gln, Gln/Gln	44 (51.1), 38 (44.2), 4 (4.7)

Tumor staging is based on the 8th edition of the Union for International Cancer Control Union for International Cancer Control TNM Classification. CRT: Chemoradiotherapy; RT: radiation therapy; SCC: squamous cell carcinoma.

Relationship between the response to ICT and possible related factors. Possible factors affecting the response to ICT are summarized in Table IV. There were no significant differences in DNA repair gene polymorphisms (*ERCC1, XRCC1, XPD312,* and *XPD751*) between the ICT non-responders and responders. There were no significant relationships between polymorphic variants and ICT response and no significant differences in sex, alcohol or tobacco consumption, squamous cell carcinoma differentiation, or TNM classification between the ICT-responders and ICT non-responders. However, the ICT responder group had significantly fewer patients aged ≥67 years and significantly fewer pathological high-risk factors.

Discussion

Chemoselection using ICT is based on the concept that ICT responders might have the opportunity for organ preservation as well as favorable prognosis (1, 2, 23-26). In the present study, HPC patients who responded to ICT had better OS than ICT non-responders. In addition, ICT responders had a higher rate of laryngeal preservation. These results suggest that TPF-based ICT is beneficial for chemoselection in HPC patients and ICT responders have better survival outcomes and organ preservation. The ICT responder group tended to include patients who received 2 or more cycles of ICT (p=0.07). The patients who underwent multiple ICT had

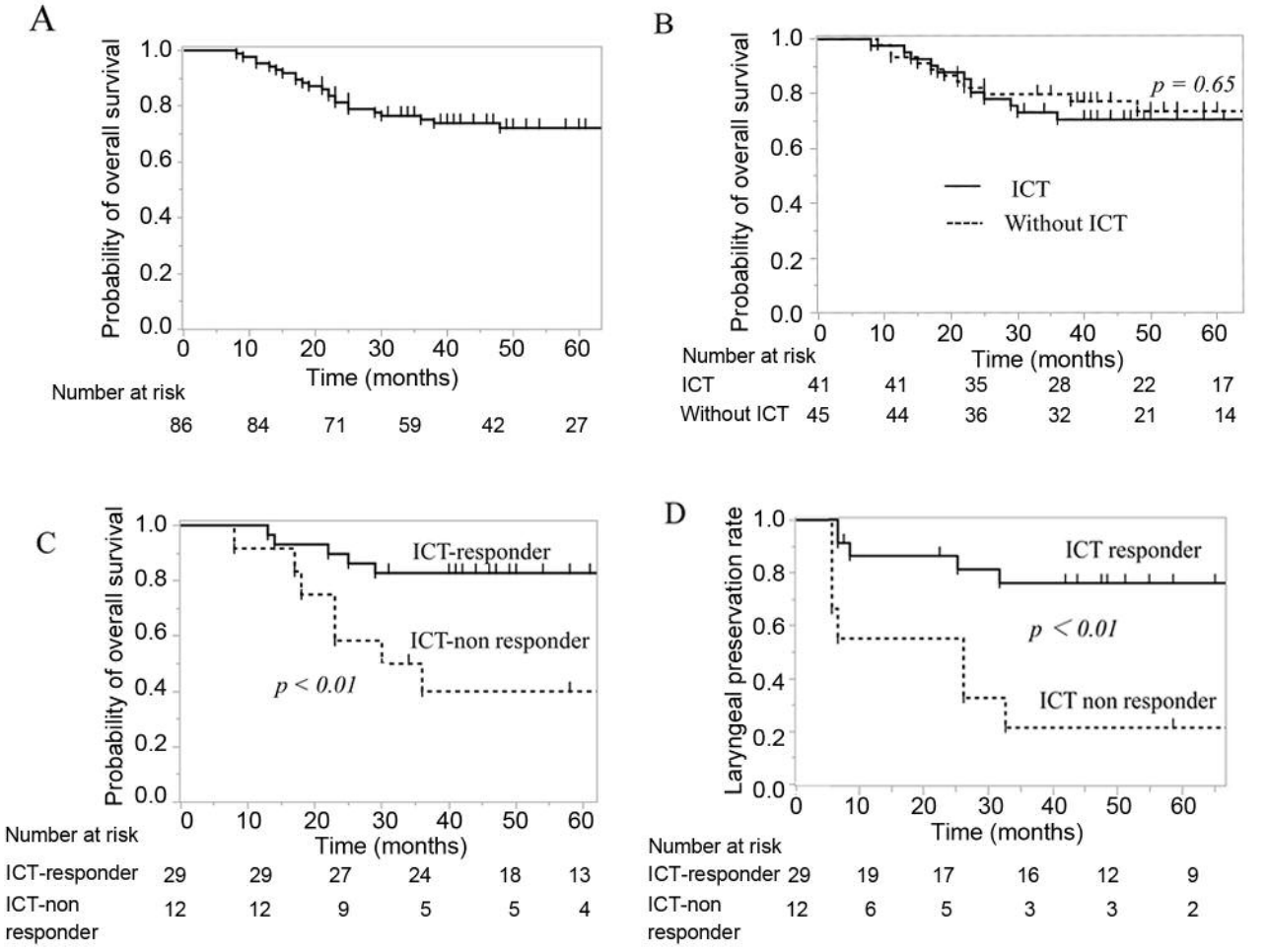

Figure 2. *Kaplan–Meier estimation of cumulative overall survival. The log-rank test was used to assess survival differences between two groups. A. The 5-year CS rate was 72.2% in all 86 hypopharyngeal patients. B. The 5-year CS rates were not different between patients with ICT and without ICT (71.3% vs. 74.0%, respectively; p=0.65). C. The 5-year CS rate in ICT responders was significantly better than that in the ICT non-responders (86.0% vs. 37.0%, respectively; p<0.01). D. Laryngeal preservation ratio in ICT responders and ICT non-responders. Laryngeal preservation rate (3-year and 5-year, 76.3%) in ICT responders was higher than that (3-year and 5-year, 22.2%) in ICT non-responders. CS: Cumulative overall survival; ICT: induction chemotherapy.*

strong preference for organ preservation and showed some response to ICT close to PR in the RECIST criteria. The majority of previous reports on ICT involved 2 or 3 cycles of ICT. Different from Caucasian patients, Japanese patients are sometimes not able to tolerate multiple ICT due to severe toxicity. However, repeated ICT may have some beneficial effect for them in achieving significant size reduction of cancerous lesions. Further study is needed to clarify this.

DNA repair pathways, namely nucleotide excision repair and mismatch repair, are candidates for predicting chemosensitivity (10, 11). However, our results failed to demonstrate a relationship between DNA repair gene polymorphisms *(ERCC1*, *XPD*-312, *XPD*-751, and *XRCC1)* and ICT response. The mRNA expression levels of DNA repair genes in blood samples correlated with corresponding gene expression levels in cancer tissue (27). However, DNA repair gene polymorphisms are related to mRNA stability and reduced DNA repair capacity, and may confer anti-tumor activity on platinum-based chemotherapy and irradiation. Gene–environment and gene–gene interactions and the combined effects of multiple SNPs in several genes might be more effective outcome predictors than single SNPs alone (28, 29). Moreover, DNA repair gene polymorphisms vary according to race. Allele and genotype frequencies of four DNA repair genes in HPC patients and controls were similar and were consistent with phase 3 data from the 1000 genomes project in a Japanese cohort. However, the data in the present study differed from the data in European

Table IV. *ICT response according to clinical and genomic factors (n = 41).*

Characteristic	ICT response			
	Non-responder	Responder	Odds ratio (95% CI)	*p*-Value
Overall patients				
	12 (29.3)	29 (70.1)		
Age (years)				
<66	3 (25.0)	18 (62.1)	0.20 (0.05-0.92)	0.03
≥67	9 (75.0)	11 (37.9)		
Gender				
Male	11 (91.7)	26 (89.7)	0.79 (0.07-8.43)	0.84
Female	1 (8.3)	3 (10.3)		
Alcohol				
Current	7 (58.3)	21 (72.4)	0.53 (0.13-2.17)	0.19
Ever+never	5 (41.7)	8 (27.6)		
Tobacco				
Current	7 (58.3)	15 (51.7)	1.30 (0.34-5.09)	0.70
Ever+never	5 (41.7)	14 (48.3)		
SCC differentiation				
Poorly differentiated	4 (33.3)	4 (13.8)	0.32 (0.06-1.58)	0.16
Moderately/Well differentiated	8 (66.7)	25 (86.2)		
Tumor subsite				
ps	8 (66.7)	23 (79.3)	1.92 (0.43-8.58)	0.39
pc+pw	4 (33.3)	6 (20.7)		
Clinical T classification				
T1, T2, T3	9 (75.0)	19 (65.5)	0.63 (0.13-2.88)	0.55
T4	3 (25.0)	10 (34.5)		
Clinical N classification				
N0, N1	5 (41.7)	10 (34.5)	1.36 (0.34-5.39)	0.67
N2, N3	7 (58.3)	19 (65.5)		
Clinical stage				
1,2	0 (0)	4 (13.8)	N/A	0.09
3,4	12 (100)	25 (86.2)		
Number of polymorphic variants				
0	6 (50.0)	8 (27.6)	0.38 (0.09-1.54)	0.17
1, 2, 3	6 (50.0)	21 (72.4)		
ICT course				
1 course	11 (91.7)	18 (62.1)	6.72 (0.76-59.5)	0.07
>2 courses	1 (8.3)	11 (37.9)		
Definitive treatment				
Surgery	7 (58.3)	13 (44.8)	1.72 (0.44-6.72)	0.43
CRT	5 (41.7)	16 (55.2)		
Post treatment high-risk factor				
Risk factor (–)	5 (41.7)	13 (44.8)	reference	
Clinical residual neck disease	0 (0)	7 (24.1)	N/A	0.29
Pathological high-risk factor	7 (58.3)	9 (31.0)	5.83 (1.30-26.2)	0.03
Polymorphism				
ERCC1 (rs3212986)				
Com	11 (91.7)	21 (72.4)		
Pol	1 (8.3)	8 (27.6)	0.18 (0.02-1.64)	0.15
XPD-312 (rs1799793)				
Com	12 (100)	27 (93.1)		
Pol	0 (0)	2 (6.9)	N/A	0.46
XPD-751 (rs13181)				
Com	12 (100)	27 (93.1)		
Pol	0 (0)	2 (6.9)	N/A	0.46
XRCC1 (rs25487)				
Com	6 (50)	15 (51.7)		
Pol	6 (50)	14 (48.3)	1.67 (0.45-6.13)	0.92

Com: Common homozygotes; CRT: chemo-radiotherapy; N/A: not available; Pol: heterozygotes and polymorphic homozygotes; ps: piriform sinus; pc: posterior cricoid; pw: posterior wall; SCC: squamous cell carcinoma.

countries, especially with relation to *XPD*-312 and *XPD*-751, with heterozygotes and polymorphic homozygotes being more common in European countries than in Japan (16, 30, 31). These ethnic variations might affect ICT response and survival outcome in patients following CCRT. Further prospective studies are needed involving large numbers of patients and drug effect (32, 33) under strict treatment protocols.

In conclusion, HPC patients who responded to ICT had better OS and better laryngeal preservation rates than ICT non-responders. These results demonstrate that TPF-based ICT is beneficial for chemoselection in HPC patients and that ICT responders have better survival outcomes and organ preservation. Nevertheless, DNA repair gene polymorphisms *(ERCC1*, *XPD*-312, *XPD*-751, and *XRCC1*) were not related to ICT response. Because of ethnic variations in DNA repair gene polymorphisms, further large, rigorously conducted prospective studies are needed.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this work.

Authors' Contributions

HH planned and performed the experiments, and wrote the manuscript; TI, SA and YY performed experiments; MS, HM and AG supervised the study and contributed to the writing of the manuscript; SA, HK, KT, JU, AK, and SK, performed clinical treatment, interpreted data and reviewed the manuscript.

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