Cancer Cell International

RESEARCH Open Access

Olaparib enhancing radiosensitization and anti-metastatic effect of oral cancer by targeting IL-17A signal

Chih-Chia Yu[1,2], Hon-Yi Lin[2,3], Michael W.Y. Chan[4,5,6,7], Shu-Fen Wu[4], Wen-Yen Chiou[2,3], Moon-Sing Lee[2,3], Chen-Lin Chi[8], Ru-Inn Lin[2], Feng-Chun Hsu[2], Hsuan-Ju Yang[2], Liang-Cheng Chen[2,3], Chia-Hui Chew[2,3] and Shih-Kai Hung[2,3*]

Abstract

Purpose We tested whether the PARP inhibitor, Olaparib, can effectively enhance radiosensitivity while inhibiting OSCC growth and metastasis in vitro and in vivo. Patient samples were used for survival validation.

Methods The present study investigated the effect of Olaparib and ionizing radiation (IR) on clonogenic, migratory, and invasive ability in human IR-sensitive (OML1) and IR-resistant (OML1-R) OSCC cell lines. We next explored the underlying mechanism with ELISA and a Western blotting assay. Two in vivo mouse models were established to investigate the efficacy of Olaparib combined with radiotherapy (RT) on local tumor growth and lung metastasis. IL-17 A expression was confirmed in tissue specimens of OSCC patients by immunohistochemistry.

Results We found that Olaparib, in combination with IR, substantially inhibited cell growth, migration, and invasion in vitro. Mechanistically, the Olaparib treatment significantly reduced the secretion of IL-17 A in irradiated OSCC cells by attenuating NF-κB and p38 activity. Consistently, Olaparib enhanced the radiosensitivity and, with RT, synergistically reduced both tumor growth and lung metastasis in mice. In addition, OSCC patients with high IL-17 A expression were substantially associated with an increased risk of lymph node involvement and worse survival.

Conclusions This study has highlighted that Olaparib displays radiosensitizing and antimetastatic effects by inhibiting the IL-17 A-dependent signal. Remarkably, Olaparib could provide a remarkable anticancer efficacy to improve treatment response in OSCC patients with recurrent/metastatic disease after RT.

Keywords Olaparib, Radiosensitization, Antimetastatic, IL-17A, Oral squamous cell carcinoma

*Correspondence:
Shih-Kai Hung
oncology158@yahoo.com.tw
[1]Department of Medical Research, Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Chia-Yi, Taiwan
[2]Department of Radiation Oncology, Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, NO2. Min-Sheng Road, Dalin Town, Chia-Yi, Chia-Yi 62247, Taiwan
[3]School of Medicine, Tzu Chi University, Hualian, Taiwan
[4]Department of Biomedical Sciences and Institute of Molecular Biology, National Chung Cheng University, Chia-Yi, Taiwan
[5]Epigenomics and Human Diseases Research Center, National Chung Cheng University, Min-Hsiung, Chiayi, Taiwan
[6]Center for Innovative Research on Aging Society (CIRAS), National Chung Cheng University, Min-Hsiung, Chia-Yi, Taiwan
[7]Research Center for Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[8]Department of Pathology, Chiayi Chang Gung Memorial Hospital, Chia-Yi, Taiwan

Introduction

Oral cancer is the sixth among the major ten causes of cancer mortality in Taiwan, according to the annual report of the Department of Health's 2020 Cancer Registry Report. Surgery combined with postoperative radiotherapy (RT) is commonly used on high-risk patients. Despite significant advances in diagnostic and therapeutic approaches for oral cancer, the high mortality due to locoregional recurrences and distant metastasis in patients following treatment remains a bottleneck for treating oral cancer in the clinic. In addition, poor radiosensitivity is a considerable factor leading to the failure of RT. Therefore, exploring the mechanism underlying radiosensitivity and metastasis is necessary to seek an effective therapeutic strategy for oral cancer.

Olaparib (AZD-2281, trade name Lynparza), a highly potent poly-ADP ribose polymerase (PARP) inhibitor, was recently approved by the Food and Drug Administration to treat cancers with inherent defects in their DNA repair pathways [1], including ovarian [2] and breast cancers [3]. Remarkably, it has been demonstrated to exert radiosensitization properties in various tumor types [4, 5]; it was also reported to have promising activity in patients with metastatic cancer [6], and thus Olaparib may provide a promising therapeutic strategy for oral cancer. However, the action mechanisms involved are still unclear, and more research is needed.

Recent advances have indicated that chemokines and cytokines play critical roles in cancer-related inflammation and promote the development of aggressive tumors [7, 8]. To investigate the potential novel mechanism of Olaparib, we have applied a screening approach with human cytokine antibody arrays to preliminarily identify significant alterations. We found that Olaparib could affect the Interleukin-17 A (IL-17 A) regulation in oral cancer cells. IL-17 A is one of the IL-17 family of cytokines, is expressed in cell types, and is implicated in the pathogenesis of inflammatory diseases [9]. Moreover, it has been detected in human cancers [10], and is closely associated with tumor progression and metastasis.

However, there is little data about how Olaparib modulates IL-17 A to control oral cancer progression. Therefore, our study aimed to explore Olaparib's capacity to enhance RT's antitumor and anti-metastasis effects. In addition, we further explored whether Olaparib-mediated IL17A activation underlies radiosensitivity and metastasis potential in oral cancer.

Materials and methods

Chemicals and reagents

Antibodies against IL-17 A, Phospho-p38, NF-κB-p65, and p44/42 (Erk1/2) were purchased from Cell Signaling Technology (Beverly, MA). We dissolved Olaparib (Toronto Research Chemicals, Ontario, Canada) in dimethyl sulfoxide (DMSO) and stored it at −20˚C.

Cell lines and cell culture

Parental (OML1) and acquired-radioresistant (OML1-R) cell lines were established and maintained in RPMI1640 containing 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, as previously reported [11]. The MOC2 murine OSCC cell line was cultured in IMDM//F12 containing 5% FBS, 5 ug epidermal growth factor (EGF), 40ug hydrocortisone, 5 mg/mL insulin, 1% penicillin-streptomycin.

Clinical specimens

The present study is a retrospective study with formalin-fixed paraffin-embedded tissue blocks of OSCC. All OSCC patients who underwent surgery with or without postoperative RT from January 2007 to December 2014 were identified. All tumors were staged according to the American Joint Committee on Cancer (AJCC) Staging Manual. All cancer patients initially received a surgical curative treatment followed by adjuvant therapy according to the decisions of our hospital's cancer committee. Indications of RT were pT3-4, pN+, and positive or close surgical margins (i.e., ≤1 mm).

Clonogenic assay

Cells were pre-treated with indicated concentrations of Olaparib or DMSO and irradiated at 0, 2, 4, or 6 Gy (Gy). After 7 days, cell colonies were stained with 0.05% crystal violet. We dissolved irradiated cells in destain solution (7% acetic acid, 5% methanol) and counted them at OD580 using a spectrophotometer (GeneQuant 1300, GE Healthcare, UK) to quantify cell number.

Human IL-17 A ELISA

IL-17 A in the supernatants were measured using ELISA kit (Affinity Biosciences, Cincinnati, OH, USA) according to the manufacturer's instruction.

Wound-healing assay

Cells were plated in 6-well plates to a confluence of about 80%, and then we separated them by scratching with a 200ul pipette tip. After the scratching, the cells were subjected to different treatments, and photographs were taken at 0 and 16 h in a microscope.

Transwell invasion assay

As previously described [12]. The invasion was measured using the 8 μm pore size Transwell® cell culture inserts (Corning Costar). 1×10^5 HUVECs were loaded into each 24-well insert in Olaparib or RT-treated cells in the lower chamber at 37 °C. Invaded cells were fixed with 4%

paraformaldehyde after 48 h, stained with 0.1% crystal violet, and counted.

Western blotting

Load 50ug of protein samples were separated on 12.5% SDS-PAGE gel and transferred to PVDF membrane (Millipore, Billerica, MA, USA). We incubated the membrane using primary antibodies overnight at 4 °C, followed by secondary antibodies for 1 h, and visualized using ECL reagents (Millipore, Billerica, MA).

Immunohistochemistry

We diluted primary antibodies to 1:100 in 3% BSA/PBS. According to the manufacturer's instructions, the sections of patient samples were performed using the Super Sensitive™ Polymer-HRP immunohistochemistry (IHC) detection system (Biogenex, San Ramon, CA). The staining scores were determined using the intensity score (0, 1+, and 2+) and the percentage (0-100%) of reactivity. The median IL-17 A value was applied as a cutoff value to differentiate high from low expression.

In vivo subcutaneous tumor model

MOC2 (2×10^5) cells were injected subcutaneously into the 6 weeks C57BL/6 mice. Mice were randomized into four groups: (1) vehicle, (2) a single dose of 4 Gy IR two times per week, (2) 30 mg/kg Olaparib intraperitoneal (i.p.) injection two times per week, or (4) combination treatment. Tumor volume was estimated using the formula: Tumor volume=(ab2)/2, where a and b are represented as the longest and shortest diameters of the tumor, respectively.

In vivo metastasis model

For the lung metastatic tumor model, 24 h pre-irradiated (4 Gy) or non-irradiated MOC2 cells (2×10^5) were injected into the C57BL/6 mice through the tail vein. Olaparib (30 mg/kg) was administered twice weekly by i.p. injection. Hematoxylin and eosin (H&E) staining was performed on lung tissue sections. According to the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) for Lesions in Rats and Mice classification, pathological tumor invasion was determined by the severity grade index scored as 0 (no tumor cells), 1 (<25%), 2 (≤25% ~ < 50%), and 3 (≥50%). The metastatic area was divided into five grades. The total numbers of each histologic section were added together as the index for lung metastasis.

Statistical analysis

Data were analyzed using the SigmaPlot software, version 10.0 (Systat Software Inc., San Jose, CA), and SPSS software, version 12.0 (IBM, Armonk, NY). Continuous data were represented as mean ± standard deviation; Student's t-test was applied to measure the significance levels. Kaplan-Meier survival analysis was used to plot the overall and cancer-specific survival estimates. We used Cox proportional hazards regression model to examine univariate and multivariate hazard ratios for the study variables. All hazard ratios were provided with 95% confidence intervals to delineate adequate size. We defined P values of <0.05 as statistical significance.

Results

Olaparib, in combination with irradiation, enhanced tumor cell growth suppression

Cell colony formation was investigated in the OML1 and OML1-R cells under irradiation (IR) alone (0, 2, 4, or 6 Gy) or in combination with Olaparib. Both cells exhibited a decreased survival at 2, 4, and 6 Gy doses in combination with Olaparib when compared with IR alone (Fig. 1a). These results indicated that Olaparib sensitizes cells to radiation.

Olaparib and combination treatment with radiation attenuated cell migration and invasion

We assessed the effect of Olaparib or a combination of irradiation on the migration and invasion of OML1 and OML1-R cells. Compared to 4 Gy IR alone, combination treatment with Olaparib and IR significantly inhibited the migration ability in both cell lines as Olaparib alone did (Fig. 1b). Similar results were obtained in invasion assays. Olaparib efficiently inhibited OML1 and OML1-R invasion when compared to the IR-treated cells. Moreover, the combined treatment showed a more potent inhibition of the invasion capacity of oral cancer cells (Fig. 1c). These results demonstrated that combining Olaparib with IR exerted synergistic inhibitory effects on migration and invasion.

Olaparib combined with irradiation reduces IL-17 A, and its expression rescued Olaparib-mediated inhibitory effects on cell migration

Characterization of the cytokine secretion profile in OML1 and OML1-R cells treated with Olaparib, IL-17 A was identified as the significantly differentially expressed chemokine; a substantial decrease was seen in Olaparib-treated cells (data not shown). We subsequently used ELISA to confirm the effects of Olaparib on the secretion of IL-17 A. As a result, IL-17 A levels decreased in OML1 and OML1-R cells following the Olaparib treatment when compared with IR. In contrast, combined treatment resulted in a more substantial decrease in IL-17 A (Fig. 2a). To validate the critical role of IL-17 A in controlling the migration ability of Olaparib-treated oral cancer cells, we investigated the effects of Olaparib or combined treatment with IR and with or without IL-17 A on the migration ability of OML1 and OML1-R cells. After

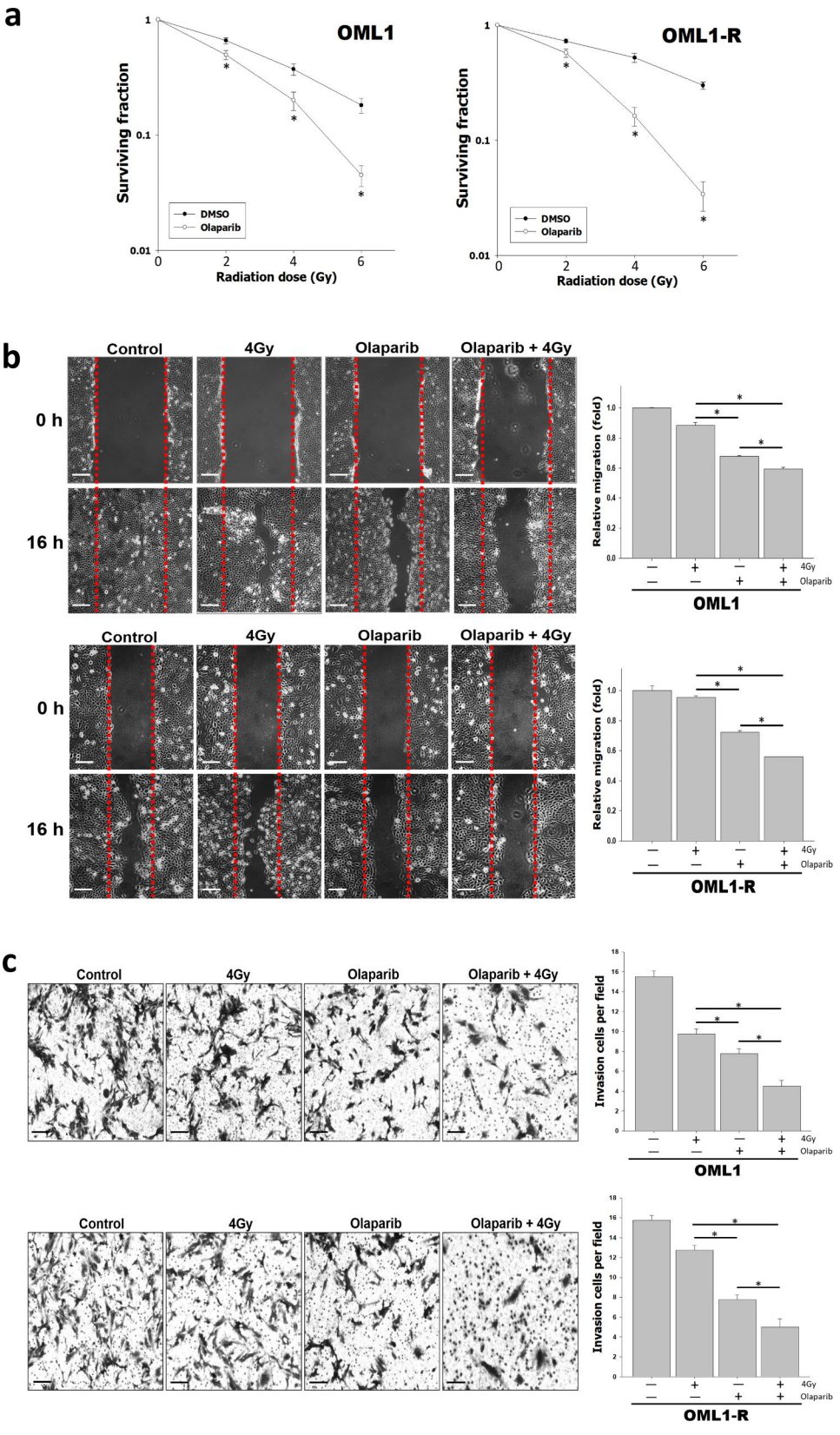

Fig. 1 Combination treatment with Olaparib and IR inhibited cell proliferation, migration, and invasion in OML1 and OML1-R cells. (**a**) Effect of Olaparib and IR on OSCC cell colony formation. Cells were exposed to IR (0–6 Gy) with or without Olaparib (12 μm for 1 h) and cultured for seven days. (**b**) Visible assessment and quantitative analysis of cell migration by wound-healing assay. (Scale bars = 100 μm) (**c**) Visible assessment and quantitative analysis of cell invasion by Transwell assay. (Scale bars = 100 μm). Data (mean ± SD) were representative of three independent experiments. *$p < 0.05$

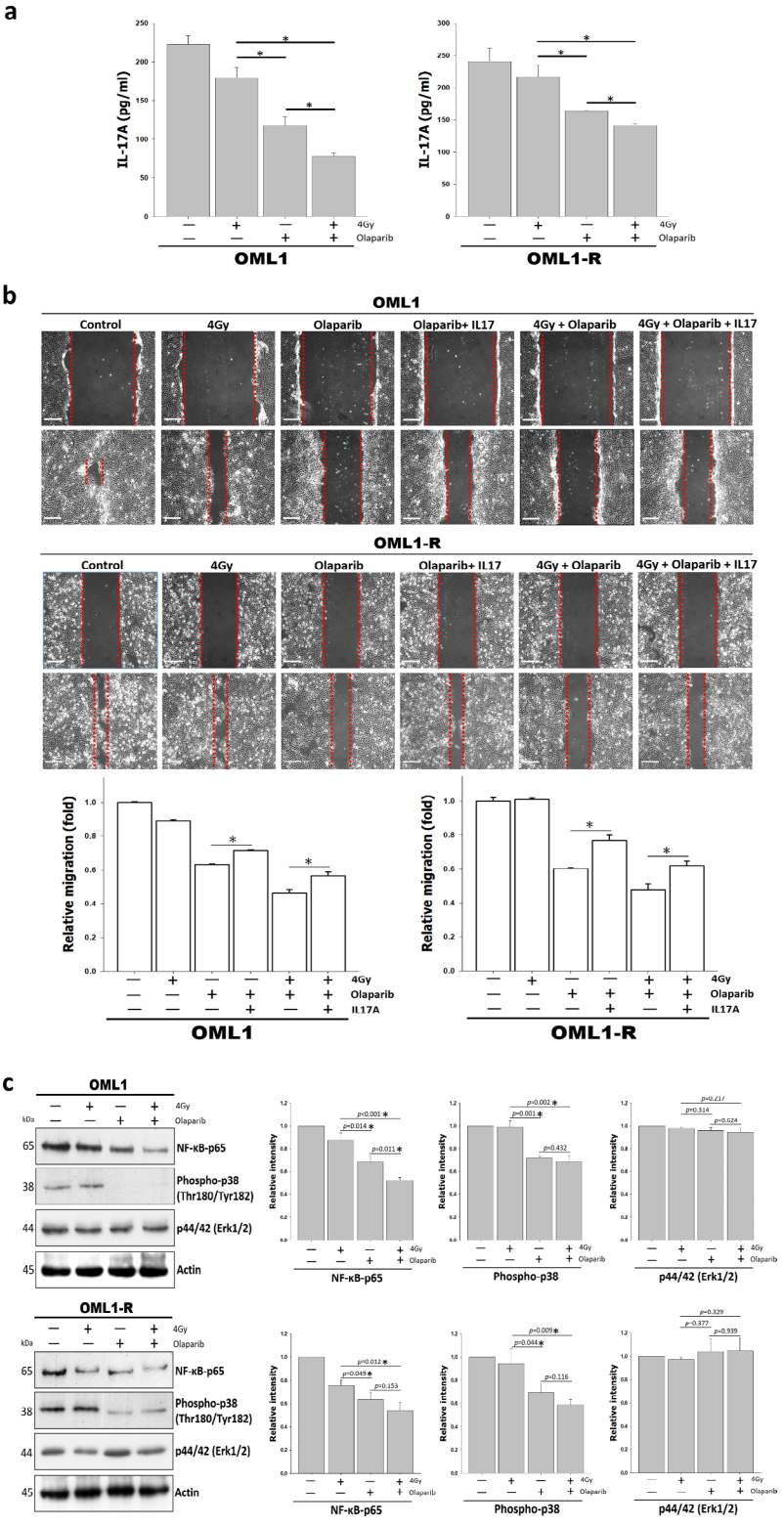

Fig. 2 Effect of Olaparib on IL17A-mediated NF-κB and p38 expression in irradiated OML1 and OML1-R cells. (**a**) The secretion of IL-17 A was measured with ELISA. (**b**) Wound-healing assay and corresponding quantitative analysis evaluating the migration activity of OML1 and OML1-R cells cultured with Olaparib or IR in the absence or presence of IL-17 A (200ng/ml) after 16 h. Quantification data were also indicated. (Scale bars = 100 μm) (**c**) Protein levels of NF-κB -p65, phospho-p38 and p44/42 (Erk1/2) were analyzed in the cell lysates using Western blotting. Blots band were analyzed using NIH ImageJ software. β-actin was used as a control for normalization of expression. Data (mean ± SD) were representative of three independent experiments. *$p < 0.05$

adding 200 ng/mL of IL-17 A to the conditioned medium, we observed that IL-17 A partially rescued the migration ability of Olaparib- or combination-treated cells (Fig. 2b). Our results suggest that Olaparib decreases IL-17 A levels, which can be employed as a biomarker for predicting response to Olaparib-based therapy in oral cancer.

Olaparib mainly mediates NF-κB and p38 in modulating IL-17 A-induced metastasis inhibition and the RT enhancement effect

IL-17 A has been reported to promote cancer metastasis, potentially through NF-κB or p38 pathway activation [11, 13]. We found that either Olaparib or combination treatment with radiation significantly decreased NF-κB and p38 expression, while control and IR-treated did not affect p38 and NF-κB (Fig. 2c). Besides, IL-17 A has also been shown to induce Epithelial-Mesenchymal Transition (EMT) by activating the Erk signaling, which promotes the gallbladder cancer metastatic process [14]. We did not find substantial differences in the levels of Erk between these two cell lines under untreated or treated conditions (Fig. 2c). Our data indicated that both NF-κB and p38 were crucial in Olaparib-mediated IL-17 A on cancer cell sensitivity to RT and anti-metastatic effect.

Olaparib enhanced the efficacy of RT to reduce tumor growth and lung metastasis by murine oral cancer cells in vivo

To test for combined antitumor effects of Olaparib and radiation, we implanted MOC2 tumors into the C57BL/6 mice. Although the Olaparib-treated tumor was slightly larger than the vehicle and RT groups, these differences were not statistically significant. However, combining Olaparib and RT reduced tumor growth dramatically ($p<0.002$; Fig. 3a). The expression of IL-17 A from tumors of each group in the in vivo study was evaluated by IHC staining to assess the effects of the treatments. We observed a significant decrease in IL-17 A expression with combination treatment compared to vehicle and either single agent (Fig. 3b-c).

Next, we determined whether Olaparib and RT co-treatment decreased metastasis in vivo using the C57BL/6 mice model. Olaparib treatment effectively blocked lung metastasis of MOC2 cancer cells in mice;

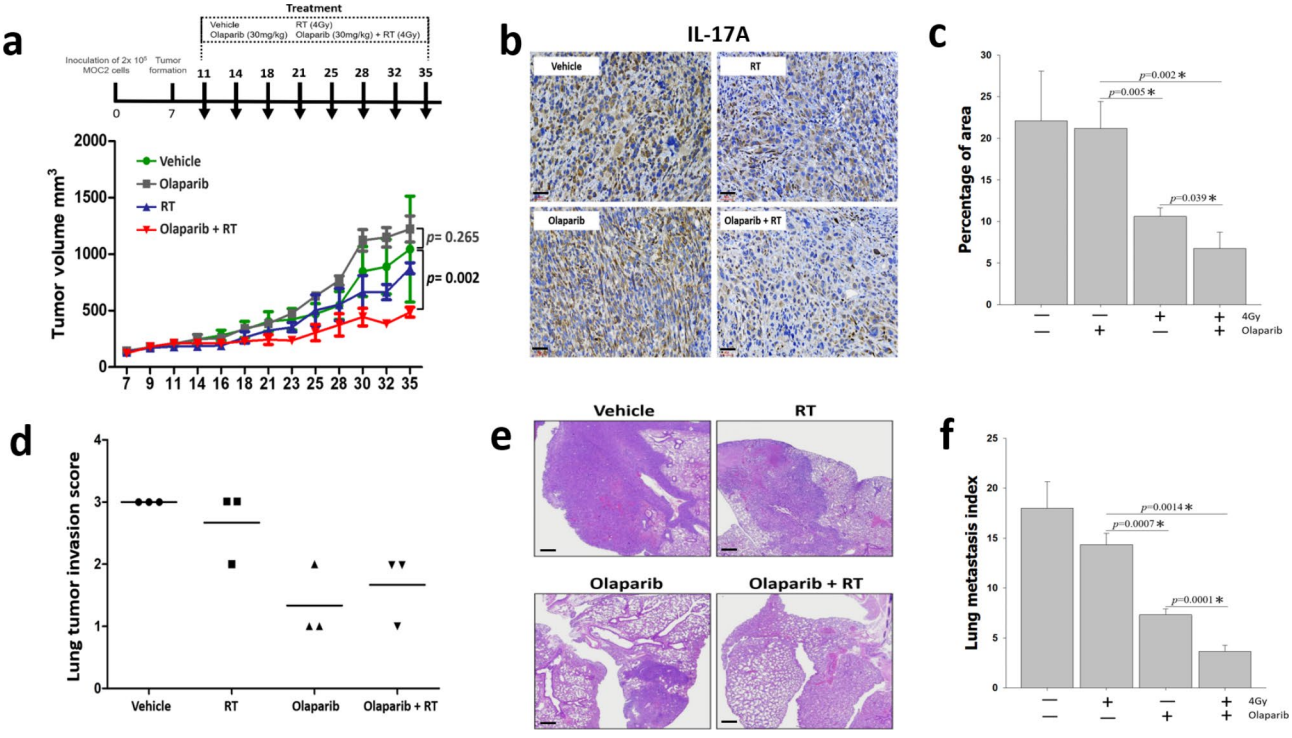

Fig. 3 Olaparib enhanced the efficacy of RT treatment to reduce tumor growth and spontaneous lung metastasis in two murine tumor models. (**a**) Schematic diagram of cell injection and treatment schedule (upper panel). Murine oral cancer MOC-2 cells were inoculated in the C57BL/6 mice and divided into four groups. Animals were treated with vehicle, IR alone (4 Gy), or with a combination of Olaparib and IR. As represented on the graph, Tumor sizes were measured three times weekly with a caliper, and the tumor volumes were calculated. (**b**) Immunohistochemical (IHC) image of IL-17 A in xenograft tumors. (**c**) Quantified analysis of the IHC images in Fig. 3b for IL-17 A using the NIH Image J software (Scale bars = 40 μm). (**d**) Scheme for Olaparib and RT treatment for a metastasis mouse. The MOC-2 cells were irradiated with 4 Gy and then were tail vein injected into C57BL/6 mice. Mice were treated with 30 mg/kg of Olaparib twice per week for 35 days via intraperitoneal injection. The graphical data represent the average tumor invasion score. (**e**) HE staining of lung specimen in xenograft tumors (Scale bars = 80 μm) and (**f**) lung metastasis index of the Fig. 3e. Data are represented as mean ± S.D. of three independent experiments. *$p<0.05$

its combination with RT showed a higher metastasis suppression than RT alone (Fig. 3d). Mice lung tissue sections from different groups were stained and evaluated using H&E staining and digital image quantification (Fig. 3e-f). Our data suggest that Olaparib, in combination with RT, inhibits spontaneous tumor growth and lung metastasis in vivo.

Strong expression of IL-17 A is associated with lymph node metastasis and poor prognosis in OSCC

We examined 122 tumors taken from patients diagnosed with OSCC. IHC staining revealed IL-17 A-positive staining mainly expressed in the cytoplasm of the tumor cells (Fig. 4a). Table 1 shows the relationship between low and high IL-17 A expression and clinical parameters. The result revealed that IL-17 A expression was significantly correlated with nodal involvement (i.e., pN+; $p=0.026$), while there was no significant association between other clinical–pathological characteristics ($p>0.05$). Kaplan-Meier survival analysis showed that patients with a higher expression of IL-17 A had a substantially poorer overall survival (OS; $p=0.011$) and cancer-specific survival (CSS; $p=0.026$; Fig. 4b) than those with a lower expression. Regarding 5-year OS, Kaplan-Meier survival analysis found that IL-17 A, age, pT, pN, extracapsular extension of lymph node (LN), lymphatic invasion, and perineural invasion were prognostic factors (Table 2). For 5-year CSS, Kaplan-Meier survival analysis demonstrated that IL-17 A, pN, margin invasion, extracapsular extension of LN, and lymphatic invasion were prognostic indicators (Table 2). Next, we used Cox proportional hazard regression to conduct univariate and multivariate analyses for OS and CSS. On multivariate analysis, IL-17 A ($p=0.008$) and perineural invasion ($p=0.01$) were statistically significantly correlated with OS (Table 3). These data suggested that higher IL-17 A expression may be associated with lymph node metastasis and poor survival.

Discussion

Cancer progression/recurrence and metastasis are substantial problems in oral cancer patients with adjuvant RT. Experimental and clinical observations indicate that radiation can facilitate tumor metastasis in some cancer cells [15, 16], the leading cause of disease progression and mortality following treatment with RT. Therefore, alternative strategies to improve RT efficiency to prevent metastatic dissemination are urgently needed. Olaparib is the first PARP inhibitor approved for patients, demonstrating good safety and significantly prolonged survival for cancer patients. Additionally, evidence has suggested that

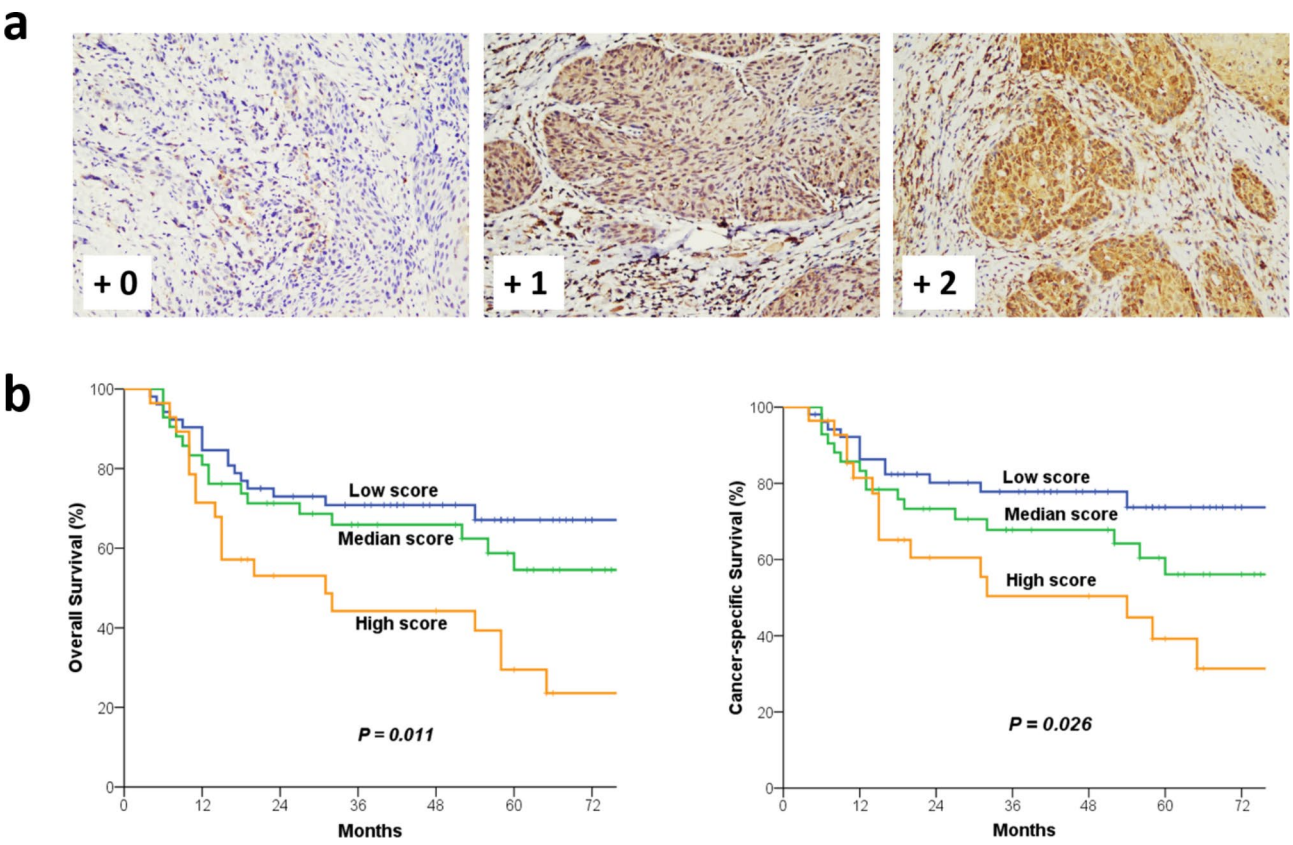

Fig. 4 The prognostic value of IL-17 A expression in OSCC. **(a)** Representative micrographs demonstrated the IHC scores of IL-17 A expression in OSCC tissues (magnification, x200). **(b)** Kaplan-Meier curves showing overall survival and cancer-specific survival rates in OSCC based on the expression of IL-17 A

Table 1 Association between IL-17 A expression in OSCC and clinicopathologic characteristics

	IL-17 A				
	Low Score (≤ 50) ***n* = 94**		***High Score (> 51)*** ***n* = 28**		***p* value**
Age					
≦55	47	50.0%	12	42.9%	0.51
> 55	47	50.0%	16	57.1%	
Gender					
Male	89	94.7%	27	96.4%	0.71
Female	5	5.3%	1	3.6%	
pT					
pT1	24	25.5%	4	14.3%	0.56
pT2	28	29.8%	9	32.1%	
pT3	15	16.0%	4	14.3%	
pT4	27	28.7%	11	39.3%	
pN					
pN0	66	70.2%	17	60.7%	0.026
pN1	8	8.5%	5	17.9%	
pN2	19	20.2%	3	10.7%	
pN3	1	1.1%	3	10.7%	
pStage					
pStage I	20	21.3%	3	10.7%	0.59
pStage II	19	20.2%	5	17.9%	
pStage III	14	14.9%	5	17.9%	
pStage IV	41	43.6%	15	53.6%	
Margin invasion					
No	85	90.4%	22	78.6%	0.09
Yes	9	9.6%	6	21.4%	
Bone invasion					
No	80	85.1%	21	75.0%	0.21
Yes	14	14.9%	7	25.0%	
Extracapsular extension of LN					
No	82	87.2%	23.0	82.1%	0.49
Yes	12	12.8%	5	17.9%	
Neck soft tissue involvement					
No	91	96.8%	26	92.9%	0.35
Yes	3	3.2%	2	7.1%	
Lymphatic invasion					
No	84	89.4%	23.0	82.1%	0.31
Yes	10	10.6%	5	17.9%	
Vascular invasion					
No	88	93.6%	25	89.3%	0.44
Yes	6	6.4%	3	10.7%	
Perineural invasion					
No	65	69.1%	22.0	78.6%	0.33
Yes	29	30.9%	6	21.4%	
Submandibular Gland Involvement					
No	92	97.9%	27	96.4%	0.55
Yes	2	2.1%	1	3.6%	
Skin involvement					
No	87	92.6%	27.0	96.4%	0.41
Yes	7	7.4%	1	3.6%	
Radiotherapy					
No	24	25.5%	7	25.0%	0.95
Yes	70	74.5%	21	75.0%	

Table 1 (continued)

	IL-17 A				
	Low Score (≤ 50) *n* = 94		*High Score (> 51)* *n* = 28		*p* value
Chemotherapy					
No	41	43.6%	11	39.3%	0.68
Yes	53	56.4%	17	60.7%	

combining RT with PARP inhibitor could be a synergistic antimetastatic efficacy in certain cancer cell lines, including melanoma [17] and cervical cancer [18], by inhibiting migration [19] .

Although Olaparib has been shown to inhibit proliferation and invasion abilities in oral cancer [20], there may be a synergistic effect that inhibits oral cancer metastasis when combined with radiation is less clear. In the present study, we first used parental OML1 cells and radioresistant OML1-R cells to assess the radiosensitizing effect of Olaparib. We showed that Olaparib, combined with IR, substantially decreased the growth rate when compared with IR-alone control cells. This result indicated that Olaparib enhanced the radiosensitivity of oral cancer cells. In addition, it is necessary to evaluate Olaparib's combining effects with irradiation further and test whether it is an effective treatment for cancer metastasis. We showed that treatment with Olaparib had noticeably an inhibiting effect on the migration and invasion of irradiated cells, confirming the experimentally in OML1 and OML1-R cells.

Since PARP is involved in regulating inflammatory processes, the inhibition of PARP has also been demonstrated as an immunomodulator to treat various types of inflammatory diseases [21, 22]. Besides, the immunomodulatory properties of PARP inhibitors have also been exploited to enhance antitumor immunity [23]. To clarify the molecular mechanism of an immune-related protein involved in the radiosensitization of Olaparib, we applied the cytokine profile array approach. We observed a substantial change in IL-17 A in Olaparib-treated cells. IL-17 A is a pro-inflammatory cytokine that has multifaceted roles in tumor formation. It is now known that IL-17 A may play pro-tumor [24] or antitumor properties [25] in different tumor contexts. It has been reported to be highly expressed in tongue squamous cell carcinoma (TSCC) and positively correlated with tumor metastasis and poor outcome [26]. Several studies have shown that PARP inhibition exhibit markedly reduced IL-17 A, which correlates with improved inflammatory disease symptoms. For example, in adjuvant-induced arthritis (AIA) mice experiments, PARP inhibition 5-aminoisoquinoline (5-AIQ) treatment reduces IL-17 A, NF-κB-p65 release levels and attenuates the severity of AIA [27].

Similarly, the PARP inhibitor 3-aminobenzamide (3-AB) is reported to ameliorate the outcome of ischemic stroke and may be critically involved in decreasing IL-17 [28]. However, Olaparib-mediated inhibition of IL-17 A enhances potential antimetastatic and radiosensitizing activities in oral cancer has not been studied yet. In the present study, we subsequently confirmed that Olaparib could decrease IL-17 A in OML1 and OML1-R cells, especially in combination with RT.

PARP inhibitor treatment suppresses cancer cell migration and invasion [20]. Our data showed that combining Olaparib with IR significantly inhibited migration/invasion ability by OML1 and OML1-R cells. IL-17 A has been shown to promote metastatic progression [29]. To investigate the functional effects of Olaparib on the migration of oral cancer cells could be counteracted by blocking IL-17 A mediated signal transduction. We observed that in both cells, adding IL-17 A abrogated Olaparib-reduced migration effectively. Besides, evidence indicates that NF-κB and MAPK pathways are involved in the induction of IL-17 A expression [13, 24]. PARP inhibitors exert anti-inflammatory properties and anticancer effects by inhibiting the NF-κB and MAPK signaling [30, 31]. The present study found that Olaparib treatment and irradiation effectively decreased the IL-17 A level, which enhanced the radiosensitivity and inhibited migration and invasion of oral cancer cells by downregulating NF-κB and p38 activation. We designed in vivo subcutaneous tumor model utilizing murine oral cancer MOC2 cells to determine the synergistic antitumor effect of Olaparib and RT when compared with single-agent treatments. Our studies revealed that combining Olaparib with RT resulted in a more considerable reduction in tumor volume than single-agent treatments. This founding was like to be linked to the decreased expression of IL-17 A.

Metastasis is chiefly responsible for poor OSCC outcomes of treatment. Our metastatic mouse model demonstrated that Olaparib enhances RT's effect on preventing metastasis. We observed a reduction of lung metastasis after Olaparib treatment, especially in combining RT. Currently, PARP inhibitors are being established as monotherapies and within combination therapies for cancer patients with advanced or metastatic [31, 32]. The results revealed that Olaparib enhances RT's therapeutic efficiency and reduces metastasis.

We evaluated the clinical significance of IL-17 A expression in 122 pathological stage I-IV oral squamous

Table 2 Analysis of the prognostic factors for 5-year overall survival (OS), cancer-specific survival rate (CSS) in OSCC patients

	5-year overall survival rate (%)	*p* value	5-year cancer-specific survival rate (%)	*p* value
IL-17 A				
≤ 50	60.5	0.007	64.6	0.033
> 51	29.5		39.2	
Age				
≤ 55	62.4	0.015	63.5	0.125
> 55	43.4		53.6	
Gender				
Male	52.7	0.955	56.5	0.817
Female	66.7		66.7	
pT				
pT1	54.3	0.034	56.4	0.053
pT2	66.4		72.6	
pT3	35.1		55.7	
pT4	42.4		48.2	
pN				
pN0	62.2	0.003	70.3	< 0.001
pN1	42.2		42.2	
pN2	31.8		35.4	
pN3	0		0.0	
pStage				
pStage I	64.3	0.053	67.4	0.073
pStage II	65.4		71.6	
pStage III	52.4		65.5	
pStage IV	41.0		46.5	
Margin invasion				
No	55.7	0.057	62.0	0.039
Yes	31.1		33.9	
Bone invasion				
No	54.7	0.525	62.1	0.192
Yes	42.2		42.2	
Extracapsular extension of LN				
No	58.5	< 0.001	64.6	< 0.001
Yes	18.3		20.9	
Neck soft tissue involvement				
No	53.1	0.330	59.4	0.181
Yes	40.0		40.0	
Lymphatic invasion				
No	56.3	0.002	62.0	0.005
Yes	26.7		34.9	
Vascular invasion				
No	54.0	0.154	60.0	0.182
Yes	33.3		40.0	
Perineural invasion				
No	59.7	0.002	63.4	0.053
Yes	36.0		46.8	
Submandibular gland involvement				
No	53.5	0.112	59.6	0.052
Yes	33.3		33.3	
Skin involvement				
No	52.4	0.962	58.8	0.755
Yes	62.5		62.5	
Radiotherapy				
No	63.8	0.647	63.8	0.784
Yes	49.2		56.9	
Chemotherapy				
No	54.1	0.989	61.4	0.941
Yes	51.3		56.1	

Table 3 Crude and adjusted hazard ratio of overall survival and cancer-specific survival in OSCC patients

	Overall survival							Cancer-specific survival						
	Crude estimate				Adjusted Estimate				Crude estimate				Adjusted Estimate			
	Coefficient	HR	95% CI		*p*-value	Coefficient	HR	95% CI		*p*-value	Coefficient	HR	95% CI		*p*-value	Coefficient	HR	95% CI		*p*-value
IL-17 A (Low, ref)	0.76	2.14	1.21	3.81	0.009	0.94	2.56	1.27	5.13	0.008	0.68	1.98	1.04	3.77	0.037	0.68	1.98	0.92	4.27	0.08
Age (≦55, ref)	0.70	2.01	1.13	3.56	0.02	0.46	1.58	0.83	3.01	0.16	0.48	1.61	0.87	2.98	0.13	0.28	1.33	0.67	2.65	0.42
pT1 (ref)					0.04					0.12					0.07					0.13
pT2	0.03	1.03	0.43	2.46	0.94	-0.33	0.72	0.29	1.77	0.47	-0.14	0.87	0.33	2.26	0.77	-0.46	0.63	0.23	1.69	0.36
pT3	0.96	2.61	1.06	6.44	0.04	0.68	1.98	0.77	5.07	0.16	0.87	2.39	0.89	6.38	0.08	0.63	1.88	0.67	5.26	0.23
pT4	0.75	2.12	0.96	4.65	0.06	0.40	1.49	0.63	3.51	0.36	0.71	2.02	0.87	4.70	0.10	0.43	1.53	0.62	3.80	0.36
pN0					0.006					0.13					0.002					0.07
pN1	0.65	1.92	0.84	4.42	0.1237	0.54	1.71	0.70	4.20	0.24	0.96	2.61	1.10	6.19	0.029	0.90	2.46	0.98	6.20	0.06
pN2	1.01	2.75	1.44	5.26	0.002	0.56	1.76	0.65	4.72	0.26	1.17	3.21	1.57	6.58	0.001	0.92	2.50	0.89	7.00	0.08
pN3	1.38	3.98	1.19	13.35	0.025	-0.81	0.44	0.08	2.62	0.37	1.71	5.53	1.60	19.06	0.007	-0.01	0.99	0.16	6.28	0.99
Extracapsular extension of LN (-, ref)	1.21	3.37	1.78	6.36	0.0002	0.79	2.20	0.70	6.96	0.18	1.25	3.50	1.75	7.01	0.0004	0.60	1.83	0.54	6.13	0.33
Lymphatic invasion (-, ref)	1.01	2.76	1.41	5.40	0.0030	0.20	1.23	0.53	2.83	0.63	1.01	2.76	1.31	5.78	0.0074	0.28	1.32	0.52	3.36	0.56
Perineural invasion (-, ref)	0.84	2.32	1.32	4.06	0.0033	0.85	2.33	1.21	4.50	0.01	0.61	1.84	0.98	3.47	0.059	0.46	1.59	0.76	3.34	0.22

HR, hazard ratio; CI, confidence interval

cell carcinoma (OSCC) patients. IL-17 A expression was significantly higher in lymph node metastasis. Additionally, Kaplan-Meier survival analysis showed that high expression of IL-17 A was associated with statistically significantly worse overall survival and cancer-specific survival. In multivariate analysis, IL-17 A has been determined as an independent predictor of overall survival in OSCC. It has been reported that IL-17 A activation is linked to carcinogenesis and progression of OSCC [26] and is regarded as a poor prognostic factor in other cancers [33]. Herein, we confirmed that increased IL-17 A is strongly associated with shorter survival, indicating that IL-17 A plays a vital role in OSCC pathogenesis and progression. Because activation of IL-17 A could activate NF-κB signal transduction, several previous studies have demonstrated that IL-17 A regulates NF-κB and cancer invasion and metastasis [24, 33]. Thus, one possible mechanism by which IL-17 A is positively correlated with the degree of activation of NF-κB signal transduction is to promote OSCC development.

Besides, our data showed that perineural invasion (PNI) was also independently prognostic of overall survival in OSCC patients. PNI has been recognized as a key pathological feature of poor survival in many malignancies [34]. In previous studies, it has also been considered an indicator of a poor prognosis in OSCC [35, 36]. Nerves, as components of the tumor microenvironment (TME), also crosstalks with the immune system, which could contribute to tumor progression via inflammation [37]. M2-like tumor-associated macrophages (TAMs) have been demonstrated to secrete various cytokines with metabolic functions, such as IL6, CCL5, and CCL18 in the hypoxic TME to enhance PNI [38]. Notably, IL-17 can be produced by Th17 cells, Tc17 cells, macrophages, and neutrophil [9, 39, 40]. The IL-17 in the TME are considered one of cancers' tumorigenesis progression and metastasis characteristics. Increased tumor-infiltrating Th17 cells and IL-17 were correlated with poor survival in patients with gastric and colorectal tumors [41, 42]. It has been suggested that the macrophage in breast tumor tissues were stained with anti-IL-17 antibodies. Therefore, infiltrating macrophage in breast tumor tissues demonstrated that it produced IL-17 and proposed a direct association between macrophage, IL-17, and breast cancer invasion [43]. However, the detailed biological and clinical role of PNI on survival should be further investigated in OSCC patients.

Our results provide a novel molecular rationale for combining Olaparib and radiotherapy and demonstrate that Olaparib promotes suppression of the IL-17 A signal, enhancing radiosensitivity and decreasing tumor growth and metastasis in OSCC. However, it is not clear whether IL-17 A-expressing immune cell population infiltrating interacts with the invasive features during

OSCC development. Moreover, in the future, it is worth investigating how combining Olaparib and radiation can modulate the antitumor immune response by infiltrating immune cells to develop a strategy for inhibiting both progression and metastasis of OSCC.

Acknowledgements
Not applicable.

Author contributions
Conceived the hypothesis and led the project: Shih-Kai Hung; Provided guidance in the study design: Michael W.Y. Chan, Hon-Yi Lin, Shu-Fen Wu; performed the experiments: Chih-Chia Yu, Ru-Inn Lin, Moon-Sing Lee, Wen-Yen Chiou; Statistical analysis: Chen-Lin Chi, Feng-Chun Hsu and Hsuan-Ju Yang; collected and prepared patient tissues: Liang-Cheng Chen, C-H Chew; Reviewed the results, interpreted the data, and wrote the manuscript: C-C Yu, H-Y Lin and S-K Hung.

Funding
The present study was supported by research grants from: 1) Buddhist Dalin Tzu Chi Hospital (DTCRD105(2)-E-09, DTCRD106 (2)-E-17, DTCRD107(2)-I-13). 2) Ministry of Science and Technology (MOST 109-2314-B-303 -014 -MY2). 3) National Science and Technology Council (NSTC 112-2314-B-303-020). 4) Buddhist Tzu Chi Medical Foundation (TCMF-A 111-10, TCMF-CP 111-11).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All animal protocols were performed according to the instructions of the Institutional Animal Care and Use Committee of National Chung Cheng University (IACUC no.1080701). Specimen analysis was approved by the ethics committee of Buddhist Dalin Tzu Chi Hospital (no. B10601019 and no. B11002010).

Competing interests
The authors declare no competing interests.

Received: 1 June 2024 / Accepted: 26 October 2024
Published online: 11 November 2024

References

1. Lee CK, Scott C, Lindeman GJ, Hamilton A, Lieschke E, Gibbs E, Asher R, Badger H, Paterson R, Macnab L, et al. <ArticleTitle Language="En">Phase 1 trial of olaparib and oral cyclophosphamide in BRCA breast cancer, recurrent BRCA ovarian cancer, non-BRCA triple-negative breast cancer, and non-BRCA ovarian cancer. Br J Cancer. 2019;120(3):279–85.
2. Audeh MW, Carmichael J, Penson RT, Friedlander M, Powell B, Bell-McGuinn KM, Scott C, Weitzel JN, Oaknin A, Loman N, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. Lancet. 2010;376(9737):245–51.
3. Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, Friedlander M, Arun B, Loman N, Schmutzler RK, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet. 2010;376(9737):235–44.
4. Gani C, Coackley C, Kumareswaran R, Schütze C, Krause M, Zafarana G, Bristow RG. In vivo studies of the PARP inhibitor, AZD-2281, in combination with fractionated radiotherapy: An exploration of the therapeutic ratio. Radiother Oncol. 2015;116(3):486–94.
5. Senra JM, Telfer BA, Cherry KE, McCrudden CM, Hirst DG, O'Connor MJ, Wedge SR, Stratford IJ. Inhibition of PARP-1 by olaparib (AZD2281) increases the radiosensitivity of a lung tumor xenograft. Mol Cancer Ther. 2011;10(10):1949–58.
6. de Bono J, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, Chi KN, Sartor O, Agarwal N, Olmos D, et al. Olaparib for Metastatic Castration-Resistant Prostate Cancer. N Engl J Med. 2020;382(22):2091–102.
7. Nisar S, Yousuf P, Masoodi T, Wani NA, Hashem S, Singh M, Sageena G, Mishra D, Kumar R, Haris M et al. Chemokine-Cytokine Networks in the Head and Neck Tumor Microenvironment. Int J Mol Sci 2021, 22(9).
8. Farc O, Cristea V. An overview of the tumor microenvironment, from cells to complex networks (Review). Exp Ther Med. 2021;21(1):96.
9. Ge Y, Huang M, Yao YM. Biology of Interleukin-17 and Its Pathophysiological Significance in Sepsis. Front Immunol. 2020;11:1558.
10. Xu C, Hao K, Yu L, Zhang X. Serum interleukin-17 as a diagnostic and prognostic marker for non-small cell lung cancer. Biomarkers. 2014;19(4):287–90.
11. Yu CC, Chan MWY, Lin HY, Chiou WY, Lin RI, Chen CA, Lee MS, Chi CL, Chen LC, Huang LW, et al. IRAK2, an IL1R/TLR Immune Mediator, Enhances Radiosensitivity via Modulating Caspase 8/3-Mediated Apoptosis in Oral Squamous Cell Carcinoma. Front Oncol. 2021;11:647175.
12. Hung SK, Yu CC, Lin HY, Chiou WY, Lee MS, Lin RI, Lu MC. Targeting PADI2 as a potential therapeutic strategy against metastasis in oral cancer via suppressing EMT-mediated migration and invasion and CCL3/5-induced angiogenesis. Clin Exp Metastasis 2024.
13. Chen Y, Kijlstra A, Chen Y, Yang P. IL-17A stimulates the production of inflammatory mediators via Erk1/2, p38 MAPK, PI3K/Akt, and NF-κB pathways in ARPE-19 cells. Mol Vis. 2011;17:3072–7.
14. Chen K, Tang H, Zhu P, Ye J, Liu D, Pu Y, Zhang L, Zhai W. Interleukin 17A promotes gallbladder cancer invasiveness via ERK/NF-κB signal pathway mediated epithelial-to-mesenchymal transition. J Cancer. 2020;11(15):4406–12.
15. Vilalta M, Rafat M, Graves EE. Effects of radiation on metastasis and tumor cell migration. Cell Mol Life Sci. 2016;73(16):2999–3007.
16. Sofia Vala I, Martins LR, Imaizumi N, Nunes RJ, Rino J, Kuonen F, Carvalho LM, Rüegg C, Grillo IM, Barata JT, et al. Low doses of ionizing radiation promote tumor growth and metastasis by enhancing angiogenesis. PLoS ONE. 2010;5(6):e11222.
17. Rodríguez MI, Peralta-Leal A, O'Valle F, Rodriguez-Vargas JM, Gonzalez-Flores A, Majuelos-Melguizo J, López L, Serrano S, de Herreros AG, Rodríguez-Manzaneque JC, et al. PARP-1 regulates metastatic melanoma through modulation of vimentin-induced malignant transformation. PLoS Genet. 2013;9(6):e1003531.
18. Ghorai A, Sarma A, Chowdhury P, Ghosh U. PARP-1 depletion in combination with carbon ion exposure significantly reduces MMPs activity and overall increases TIMPs expression in cultured HeLa cells. Radiat Oncol. 2016;11(1):126.
19. Valabrega G, Scotto G, Tuninetti V, Pani A, Scaglione F. Differences in PARP Inhibitors for the Treatment of Ovarian Cancer: Mechanisms of Action, Pharmacology, Safety, and Efficacy. Int J Mol Sci 2021, 22(8).
20. Nakamura N, Fujihara H, Kawaguchi K, Yamada H, Nakayama R, Yasukawa M, Kishi Y, Hamada Y, Masutani M. Possible Action of Olaparib for Preventing Invasion of Oral Squamous Cell Carcinoma In Vitro and In Vivo. Int J Mol Sci 2022, 23(5).
21. Wasyluk W, Zwolak A. PARP Inhibitors: An Innovative Approach to the Treatment of Inflammation and Metabolic Disorders in Sepsis. J Inflamm Res. 2021;14:1827–44.
22. Ahmad A, Olah G, Herndon DN, Szabo C. The clinically used PARP inhibitor olaparib improves organ function, suppresses inflammatory responses and accelerates wound healing in a murine model of third-degree burn injury. Br J Pharmacol. 2018;175(2):232–45.
23. Yélamos J, Moreno-Lama L, Jimeno J, Ali SO. Immunomodulatory Roles of PARP-1 and PARP-2: Impact on PARP-Centered Cancer Therapies. Cancers (Basel) 2020, 12(2).
24. Wang Y, Wu H, Wu X, Bian Z, Gao Q. Interleukin 17A promotes gastric cancer invasiveness via NF-κB mediated matrix metalloproteinases 2 and 9 expression. PLoS ONE. 2014;9(6):e96678.
25. Benchetrit F, Ciree A, Vives V, Warnier G, Gey A, Sautès-Fridman C, Fossiez F, Haicheur N, Fridman WH, Tartour E. Interleukin-17 inhibits tumor cell growth by means of a T-cell-dependent mechanism. Blood. 2002;99(6):2114–21.
26. Wei T, Cong X, Wang XT, Xu XJ, Min SN, Ye P, Peng X, Wu LL, Yu GY. Interleukin-17A promotes tongue squamous cell carcinoma metastasis through activating miR-23b/versican pathway. Oncotarget. 2017;8(4):6663–80.
27. Ahmad SF, Zoheir KM, Bakheet SA, Ashour AE, Attia SM. Poly(ADP-ribose) polymerase-1 inhibitor modulates T regulatory and IL-17 cells in the prevention of adjuvant induced arthritis in mice model. Cytokine. 2014;68(2):76–85.

28. Noh MY, Lee WM, Lee SJ, Kim HY, Kim SH, Kim YS. Regulatory T cells increase after treatment with poly (ADP-ribose) polymerase-1 inhibitor in ischemic stroke patients. Int Immunopharmacol. 2018;60:104–10.
29. Xu LL, Li ZJ, Niu XL, Deng WM. The mechanisms of IL-17A on promoting tumor metastasis. Int Rev Immunol. 2017;36(6):360–9.
30. Cseh AM, Fábián Z, Sümegi B, Scorrano L. Poly(adenosine diphosphate-ribose) polymerase as therapeutic target: lessons learned from its inhibitors. Oncotarget. 2017;8(30):50221–39.
31. Chowdhury P, Dey P, Ghosh S, Sarma A, Ghosh U. Reduction of metastatic potential by inhibiting EGFR/Akt/p38/ERK signaling pathway and epithelial-mesenchymal transition after carbon ion exposure is potentiated by PARP-1 inhibition in non-small-cell lung cancer. BMC Cancer. 2019;19(1):829.
32. Desnoyers A, Nadler M, Wilson BE, Stajer S, Amir E. Associations with response to Poly(ADP-ribose) Polymerase (PARP) inhibitors in patients with metastatic breast cancer. NPJ Breast Cancer. 2022;8(1):43.
33. Li J, Lau GK, Chen L, Dong SS, Lan HY, Huang XR, Li Y, Luk JM, Yuan YF, Guan XY. Interleukin 17A promotes hepatocellular carcinoma metastasis via NF-kB induced matrix metalloproteinases 2 and 9 expression. PLoS ONE. 2011;6(7):e21816.
34. Liebig C, Ayala G, Wilks JA, Berger DH, Albo D. Perineural invasion in cancer: a review of the literature. Cancer. 2009;115(15):3379–91.
35. Quintana D, Dedivitis RA, Kowalski LP. Prognostic impact of perineural invasion in oral cancer: a systematic review. Acta Otorhinolaryngol Ital. 2022;42(1):17–25.
36. Tai SK, Li WY, Chu PY, Chang SY, Tsai TL, Wang YF, Huang JL. Risks and clinical implications of perineural invasion in T1-2 oral tongue squamous cell carcinoma. Head Neck. 2012;34(7):994–1001.
37. Wang H, Zheng Q, Lu Z, Wang L, Ding L, Xia L, Zhang H, Wang M, Chen Y, Li G. Role of the nervous system in cancers: a review. Cell Death Discov. 2021;7(1):76.
38. Chen Z, Fang Y, Jiang W. Important Cells and Factors from Tumor Microenvironment Participated in Perineural Invasion. Cancers (Basel) 2023, 15(5).
39. Vykhovanets EV, Maclennan GT, Vykhovanets OV, Gupta S. IL-17 Expression by macrophages is associated with proliferative inflammatory atrophy lesions in prostate cancer patients. Int J Clin Exp Pathol. 2011;4(6):552–65.
40. Liu R, Lauridsen HM, Amezquita RA, Pierce RW, Jane-Wit D, Fang C, Pellowe AS, Kirkiles-Smith NC, Gonzalez AL, Pober JS. IL-17 Promotes Neutrophil-Mediated Immunity by Activating Microvascular Pericytes and Not Endothelium. J Immunol. 2016;197(6):2400–8.
41. Zhang B, Rong G, Wei H, Zhang M, Bi J, Ma L, Xue X, Wei G, Liu X, Fang G. The prevalence of Th17 cells in patients with gastric cancer. Biochem Biophys Res Commun. 2008;374(3):533–7.
42. Liu J, Duan Y, Cheng X, Chen X, Xie W, Long H, Lin Z, Zhu B. IL-17 is associated with poor prognosis and promotes angiogenesis via stimulating VEGF production of cancer cells in colorectal carcinoma. Biochem Biophys Res Commun. 2011;407(2):348–54.
43. Zhu X, Mulcahy LA, Mohammed RA, Lee AH, Franks HA, Kilpatrick L, Yilmazer A, Paish EC, Ellis IO, Patel PM, et al. IL-17 expression by breast-cancer-associated macrophages: IL-17 promotes invasiveness of breast cancer cell lines. Breast Cancer Res. 2008;10(6):R95.

Publisher's note