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Original Article

Increased Radiosensitivity of Solid Tumors Harboring *ATM* and *BRCA1/2* Mutations

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Purpose Preclinical data indicate that response to radiotherapy (RT) depends on DNA damage repair. In this study, we investigated the role of mutations in genes related to DNA damage repair in treatment outcome after RT.

Materials and Methods Patients with solid tumor who participated in next generation sequencing panel screening using biopsied tumor tissue between October 2013 and February 2019 were reviewed and 97 patients that received RT were included in this study. Best response to RT and the cumulative local recurrence rate (LRR) were compared according to absence or presence of missense, nonsense, and frameshift mutations in *ATM* and/or *BRCA1/2*.

Results Of the 97 patients, five patients harbored mutation only in *ATM*, 22 in only *BRCA1/2*, and six in both *ATM* and *BRCA1/2* ($ATM^{mt}BRCA^{mt}$). Propensity score matching was performed to select the control group without mutations ($ATM^{wt}BRCA^{wt}$, n=33). In total, 90 RT-treated target lesions were evaluated in 66 patients. Highest objective response rate of 80% was observed in $ATM^{mt}BRCA^{mt}$ lesions (p=0.007), which was mostly durable. Furthermore, the cumulative 1-year LRR was the lowest in $ATM^{mt}BRCA^{mt}$ lesions and the highest in $ATM^{wt}BRCA^{wt}$ lesions (0% vs. 47.9%, p=0.008). RT-associated toxicities were observed in 10 treatments with no significant difference among the subgroups (p=0.680).

Conclusion Tumors with *ATM* and *BRCA1/2* mutations exhibited superior tumor response and local control after RT compared to tumors without these mutations. The results are hypothesis generating and suggest the need for integrating the tumor mutation profile of DNA repair genes during treatment planning.

Key words Radiotherapy, *ATM*, *BRCA*, DNA repair, Radiosensitivity

Introduction

Radiotherapy (RT) has been widely utilized for curative or palliative care of patients with different types of cancer. Approximately 50% of patients with cancer require RT at least once during their courses of treatment, although the percentage may differ depending on medical infrastructure and availability of RT [1,2]. However, the degree and duration of response to RT varies widely and is largely dependent on the innate radiosensitivity of tumors and the delivered radiation dose [3,4]. Currently, radiosensitivity is known to be mostly dependent on the histology of the tumor [3-5]. Furthermore, it has been demonstrated that the intrinsic radiosensitivity of cancer cells is a major determinant of tumor response [6]. Certain molecular determinants involving DNA damage repair, oxidative stress, and apoptosis may affect radiosensitivity in cancer cells [7].

DNA damage repair is necessary for the survival of both tumor cells and normal cells [8]. Damaged DNA is efficiently repaired via an intricate repair mechanism. For instance, ataxia-telangiectasia mutated (ATM) plays an important role in the repair of DNA double-strand breaks (DSBs). BRCA1 and 2 are the other important DSB repair proteins predominantly involved in the homologous recombination-repair (HR) pathway. It is well-documented that defects in DNA damage repair are associated with increased risk of toxicity and secondary malignancies after RT. For example, unexpected extreme radiation reaction was first reported in a 10-year-old boy with autosomal recessive ataxia-telangiectasia (A-T) disorder (caused by a germline mutation in ATM

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kinase) who underwent conventional 30 Gy of RT and died after 8 months [9]. Increased risk of contralateral breast cancer was reported in women with germline *BRCA1/2* mutations who underwent lumpectomy and breast RT [10]. Increase in knowledge regarding unexpected radiation reaction has alerted clinicians when treating patients with the above genotypes with RT.

However, patients with somatic mutations in DNA repair genes in tumors, which occur more frequently than germline mutations, differ from patients with germline mutations, as the former do not harbor mutations in normal cells. Therefore, severe toxicities or secondary malignancies are not encountered frequently in these patients with cancer. Mutations in *ATM* and *BRCA1/2* are common in various solid tumors [11,12]. Considering that the tumor cell killing effect of RT is mainly driven by lethal DNA damage, RT may also be considered as a critical component of treatment in patients harboring mutations in genes responsible for DNA damage repair. However, clinical data regarding whether tumors with *ATM* or *BRCA1/2* somatic mutations are more sensitive to RT are scarce. Considering the increased use of multi-site palliation with advanced RT technologies, better understanding of the relationship between clinical RT response and such somatic mutations can facilitate the development of personalized RT regimens in the future.

In this study, we aimed to determine the effect of somatic *ATM* and *BRCA1/2* mutations on radiosensitivity and treatment response. In this regard, we retrospectively analyzed the medical records of patients with solid tumors that received RT and compared the in-field target lesion control rates according to the presence of *ATM* and/or *BRCA* mutations. In addition, we compared the risk of RT-related toxicities between these two groups.

Materials and Methods

1. Study cohort

Between October 2013 and February 2019, 97 patients that received RT and underwent gene-panel sequencing of the biopsied tumor were subjected to analysis. Frameshift, missense, or nonsense mutations in *ATM* and/or *BRCA1/2* were detected in 33 patients (mutation group); six patients harbored mutations in both *ATM* and *BRCA* ($ATM^{mt}BRCA^{mt}$), five patients harbored mutant *ATM* but wild type *BRCA* ($ATM^{mt}BRCA^{wt}$), and 22 patients harbored *BRCA* mutation but wild type *ATM* ($ATM^{wt}BRCA^{mt}$). As a control cohort, 33 patients were 1:1 propensity score-matched to the mutation group. Matching was performed using the variables that may maximally affect the RT response, which included age, sex, radiosensitivity, and RT dose (equivalent dose in 2 Gy [EQD2], < 45 Gy vs. ≥ 45 Gy). Radiosensitivity was subjectively defined as either radioresistant (colorectal cancer and melanoma) or radiosensitive (breast, lung, and gynecological malignancy) according to previous reports and our clinical experiences [4,13-15].

2. Radiotherapy

All patients underwent a computed tomography (CT) simulation using an appropriate immobilization device if required. The gross tumor volume was defined as the visible tumor in CT or other imaging studies such as positron emission tomography (PET) or magnetic resonance imaging (MRI). Three-dimensional conformal RT or intensity-modulated RT was applied based on the tumor location and the physician's discretion. The dose constraints for the normal organ were set according to the institutional consensus protocol and guideline of the Quantitative Analyses of Normal Tissue Effects in the Clinic (QUANTEC).

3. Follow-up and response evaluation

Initial follow-up for the patient was usually made one month after completion of RT. Patients were followed with 3-month interval during the first year and 6 months thereafter. Physical examination, basic laboratory tests, and imaging evaluations, including CT, MRI, or PET were performed during the follow-up. In-field tumor response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 by a board certified radiologist (blinded read), and classified as complete response (CR), partial response (PR), stable disease, and progression of disease (PD). For patients who underwent follow-up PET, Positron Emission Tomography Response Criteria in Solid Tumors (PERCIST) was adopted to evaluate tumor response. Objective response was defined as either CR or PR. The objective response rate was defined as the proportion of patients achieving an objective response. For bone lesions, lytic or mixed lytic-blastic lesions with identifiable soft tissue components that were considered measurable were evaluated. The primary endpoints were best response to RT and the local recurrence rate (LRR). The second endpoint was toxicity. LRR was defined as the time from RT initiation to local recurrence or last follow-up. Local recurrence was defined as the PD at the irradiated site.

4. Next generation sequencing

Targeted DNA or RNA sequencing was performed using TruSight Tumor 170 (Illumina, San Diego, CA), which detects 170 cancer-related genes [16]. We have focused on genes related to DNA damage response pathway, such as *ATM*, *BRCA1*, *BRCA2*, *TP53*, and *PALB2*. Briefly, 40 ng DNA and RNA were extracted from formalin-fixed paraffin-

Table 1. Characteristics of the 91 lesions according to mutation status

Characteristic	*ATM*^{wt}*BRCA*^{wt} (n=46)	*ATM*^{mt}*BRCA*^{wt} (n=8)	*ATM*^{wt}*BRCA*^{mt} (n=27)	*ATM*^{mt}*BRCA*^{mt} (n=10)	p-value
Age (yr)	56 (28-72)	46 (39-57)	55 (36-71)	46 (26-66)	0.023
Sex					
Male	19 (41.3)	6 (75.0)	10 (37.0)	3 (30.0)	0.238
Female	27 (58.7)	2 (25.0)	17 (63.0)	7 (70.0)	
Primary site					
Breast	1 (2.2)	1 (12.5)	2 (7.4)	2 (20.0)	0.004
Lung	18 (39.1)	0	10 (37.0)	1 (10.0)	
Gastrointestinal	18 (39.1)	3 (37.5)	8 (29.6)	4 (40.0)	
Genitourinary	9 (19.6)	1 (12.5)	5 (18.5)	3 (30.0)	
Skin	0	0	2 (7.4)	0	
Unknown	0	3 (37.5)	0	0	
Disease extent					
Metastatic	42 (91.3)	8 (100)	26 (96.3)	10 (100)	0.885
Localized	4 (8.7)	0	1 (3.7)	0	
Radiosensitivity					
Sensitive	28 (60.9)	2 (25.0)	17 (63.0)	6 (60.0)	0.286
Resistant	18 (39.1)	6 (75.0)	10 (37.0)	4 (40.0)	
Radiotherapy site					
Brain	11 (23.9)	0	6 (22.2)	1 (10.0)	0.351
Lymph node	12 (26.1)	4 (50.0)	5 (18.5)	4 (40.0)	
Bone	7 (15.2)	3 (37.5)	8 (29.6)	0	
Lung	10 (21.7)	2 (25.0)	3 (11.1)	1 (10.0)	
Liver	2 (4.3)	0	3 (11.1)	1 (10.0)	
Others	4 (8.7)	0	3 (11.1)	3 (30.0)	
Pre-RT chemotherapy					
Cytotoxic chemo	35 (76.1)	8 (100)	19 (70.4)	7 (70.0)	0.484
Target agents	5 (10.9)	0	5 (18.5)	3 (30.0)	
None	6 (13.0)	0	3 (11.1)	0	
Site of NGS performed					
Primary	30 (65.2)	2 (25.0)	17 (63.0)	4 (40.0)	0.111
Metastatic	16 (34.8)	6 (75.0)	10 (27.0)	6 (60.0)	
RT site and sequenced site matched					
No	37 (80.4)	7 (87.5)	24 (88.9)	9 (90.0)	0.826
Yes	9 (19.6)	1 (12.5)	3 (11.1)	1 (10.0)	
Type of RT[a]					
Conventionally fractionated	12 (26.1)	1 (12.5)	3 (11.1)	5 (50.0)	0.122
Hypofractionated	28 (60.9)	6 (75.0)	23 (85.2)	5 (50.0)	
SBRT	6 (13.0)	1 (12.5)	1 (3.7)	0	
EQD2 (Gy)					
< 45	17 (37.0)	2 (25.0)	19 (70.4)	4 (40.0)	0.022
≥ 45	29 (63.0)	6 (75.0)	8 (29.6)	6 (40.0)	

Values are presented as median (range) or number (%). EQD2, equivalent dose in 2 Gy; NGS, next generation sequencing; RT, radiotherapy; SBRT, streotactic body radiotherapy. [a]Conventional fractionation (1.8-2.4 Gy), hypofractionation (2.5-8 Gy), SBRT (≥ 10 Gy).

embedded (FFPE) tissue using the Qiagen All Prep DNA/RNA FFPE kit (Qiagen, Hilden, Germany). After hybridization capture-based target enrichment, pair-ended sequencing (2×150 bp) was performed using a NextSeq sequencer (Illumina) according to the manufacturer's instructions. Variant calling was performed using Illumina App-pipeline (Illumina). Variants with a total depth of at least 100× and variant allele frequency of at least 3% were included for

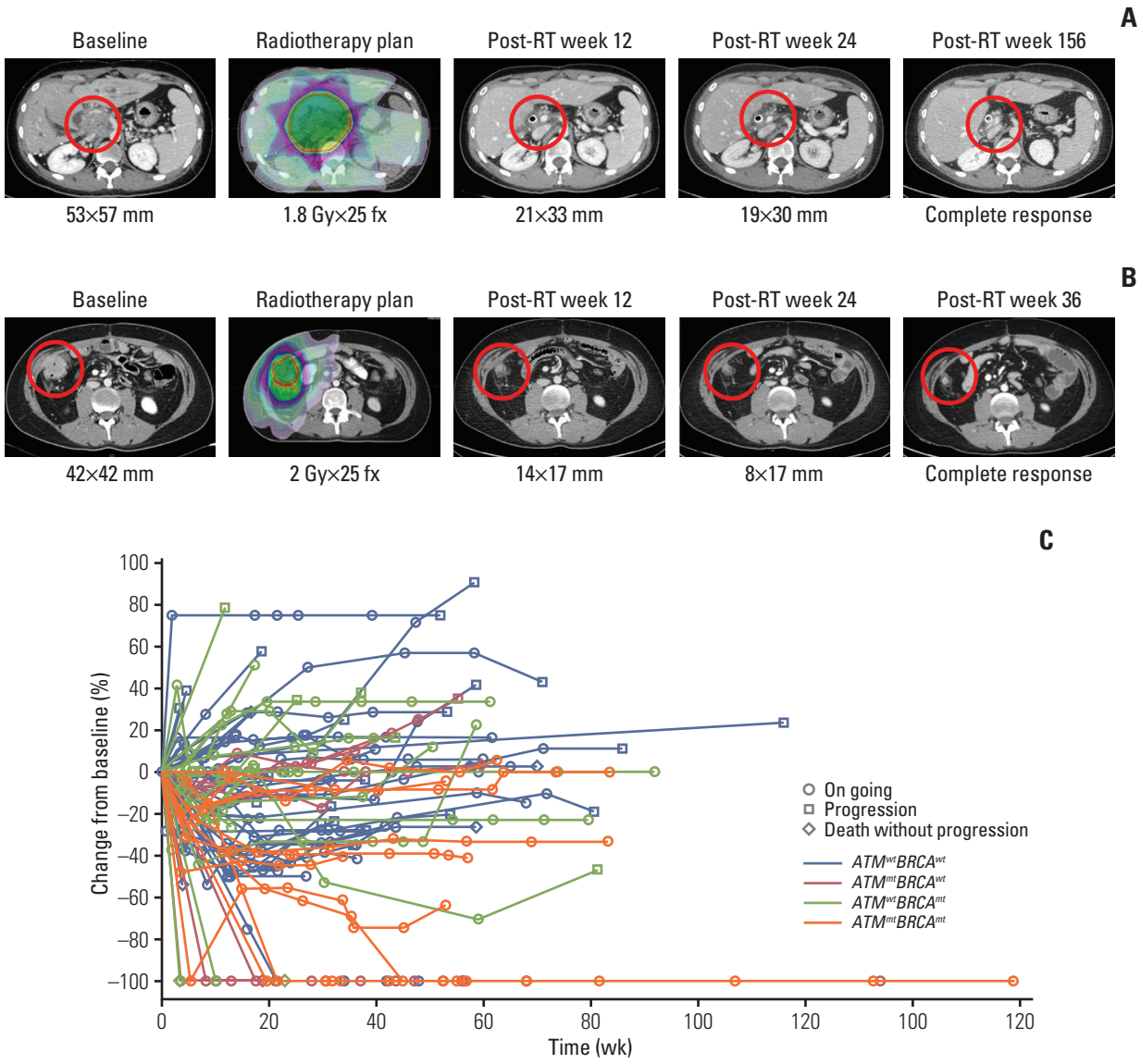

Fig. 1. Serial tumor response in patients treated with radiotherapy. (A) A representative patient diagnosed with Adenocarcinoma in Rectosigmoid Junction was initially treated with systemic chemotherapy and was referred for progression in aortocaval lymph node. A total dose of 45 Gy in 25 fractions was delivered to the lesion and the patient exhibited a marked response at 12-weeks post-radiotherapy and no recurrence at 185 weeks. A biliary stent was inserted after radiotherapy for bile drainage. (B) The same patient presented with a peritoneal metastatic lesion after 2 years and was treated with a total dose of 50 Gy in 25 fractions. The treated lesion also showed marked reduction in size and no recurrence at 48 weeks. (C) Change in the longest tumor diameter over time compared to the baseline of lesions subjected to radiotherapy.

analysis. Benign single nucleotide polymorphisms (≥ 1% frequency based population-based database) were filtered out. Variant interpretation was based on recommendations from the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists [17]. The clinical significance of mutations was classified by a four-tiered system; tier I, variants with strong clinical significance; tier II, variants with potential clinical significance; tier III, variants of unknown clinical significance; and tier IV, variants deemed benign or likely benign (S1 Table). Tier I, II, and III variants were included in our study.

5. Statistical analysis

Patient characteristics were compared using the chi-square test, Fisher exact test, Student's t test, or the analysis of variance (ANOVA) as applicable. Cumulative LRR was calculat-

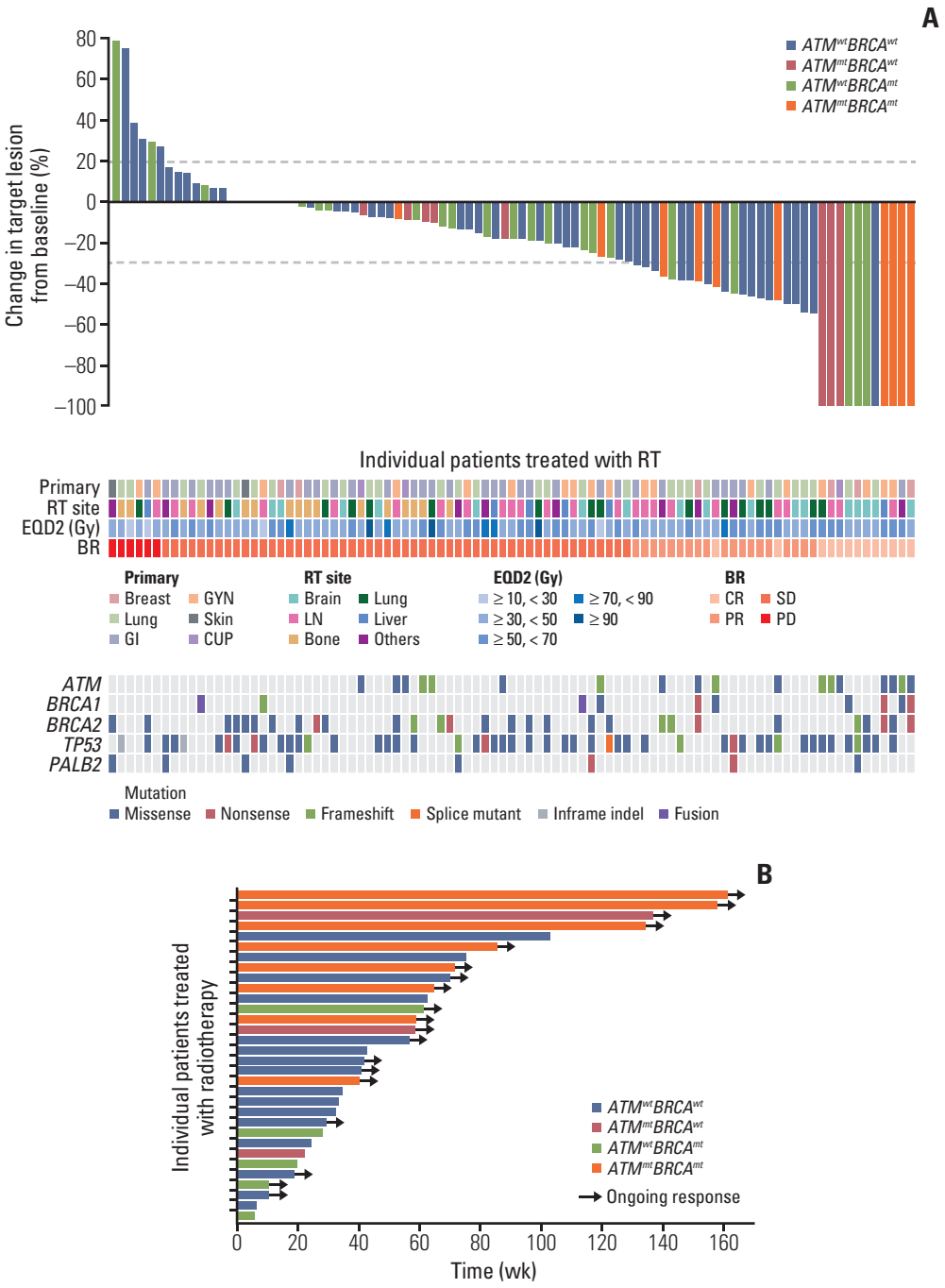

Fig. 2. Treatment response of patients treated with radiotherapy according to mutational status. (A) A waterfall plot of the percentage changes in radiotherapy-treated lesions compared to measurements in pre-radiotherapy lesions. The bottom panel represents the primary tumor site, radiotherapy (RT) site, radiotherapy dose (EQD2), best response (BR), and mutation status of *ATM*, *BRCA1*, *BRCA2*, *TP53*, and *PALB2*. The line at 20% and –30% indicate the threshold for progressive disease and objective response per *Response Evaluation Criteria in Solid Tumors* (RECIST) ver. 1.1. Two patients with partial response (PR) per RECIST exhibited a complete metabolic response according to Positron Emission Tomography Response Criteria in Solid Tumors. CR, complete response; CUP, cancer of unknown primary; GI, gastrointestinal; GYN, gynecologic; PD, progression of disease; SD, stable disease. (B) A swimmer plot representing the duration of response of patients who exhibited an objective response. Arrows indicate an ongoing response at the time of data censoring.

Table 2. Predictors of objective response after RT

Variable	Univariate		Multivariate	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Mutation group		0.023		0.016
ATM^{wt}*BRCA*^{wt}	Reference		Reference	
ATM^{mt}*BRCA*^{wt}	1.13 (0.24-5.33)	0.882	1.78 (0.34-9.48)	0.499
ATM^{wt}*BRCA*^{mt}	0.43 (0.14-1.34)	0.144	0.40 (0.12-1.29)	0.124
ATM^{mt}*BRCA*^{mt}	7.50 (1.42-39.60)	0.018	8.87 (1.56-50.45)	0.014
Age (yr)	0.98 (0.94-1.02)	0.223	-	-
Sex		0.545		-
Male	Reference			
Female	1.31 (0.54-3.17)		-	
Radiosensitivity		0.055		0.030
Sensitive	Reference		Reference	
Resistant	0.40 (0.16-1.02)		0.31 (0.11-0.89)	
Primary site		0.457		-
Breast	Reference			
Lung	0.31 (0.05-1.95)	0.210	-	-
Gastrointestinal	0.16 (0.03-1.04)	0.055	-	-
Genitourinary	0.40 (0.06-2.77)	0.353	-	-
Skin	0 (0-NA)	0.999	-	-
Unknown	0.25 (0.01-4.73)	0.355	-	-
Radiotherapy site		0.756		-
Brain	Reference			
Lymph node	0.57 (0.17-1.96)	0.374	-	-
Bone	0 (0-NA)	0.998	-	-
Lung	0.16 (0.02-1.66)	0.125	-	-
Liver	0.62 (0.16-2.42)	0.493	-	-
Others	0.53 (0.11-2.56)	0.433	-	-
Pre-RT chemotherapy		0.513		-
Cytotoxic chemo	Reference			
Target agents	1.83 (0.55-6.09)	0.324	-	-
None	1.71 (0.42-6.99)	0.456	-	-
EQD2 (Gy)		0.225		-
< 45	Reference			
≥ 45	1.72 (0.72-4.15)		-	-

CI, confidence interval; EQD2, equivalent dose in 2 Gy; NA, not applicable; OR, odds ratio; RT, radiotherapy.

ed by competing risk analysis, and comparisons were made using Gray's test. A competing event was defined as death from any cause without evidence of local recurrence. Propensity score matching was performed using MatchIt package ver. 3.0.2. A generalized linear model was used without a prespecified caliper. Different variables, such as age, sex, radiosensitivity, pre-RT chemotherapy, radiation dose (EQD2), and type of RT modality, were included for matching. Primary and irradiated sites were excluded since they were highly diverse compared to the number of patients matched. Logistic regression was performed to identify the significant predictors of objective response. Variables with $p < 0.1$ in the univariate analysis were included in the multivariate analysis. Two-sided $p < 0.05$ was considered statistically significant. Statistical analyses were conducted using IBM SPSS Statistics ver. 24.0 (IBM Corp., Armonk, NY), GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA), and R ver. 3.6.0 (https://www.r-project.org).

Results

1. Patient characteristics

Baseline characteristics of patients in the mutation group

and wild type group before and after propensity score matching are shown in S2 Table. The median RT dose (EQD2) delivered was 44.7 Gy (range, 11.5 to 125 Gy) and it was dichotomized to < 45 Gy and ≥ 45 Gy. The characteristics were well balanced after matching (S2 Table). Patients in the mutation group were further stratified by *ATM* and *BRCA* mutation status (S3 Table). No significant differences in baseline characteristics were observed between the four groups: *ATM*^{wt}*BRCA*^{wt}, *ATM*^{mt}*BRCA*^{wt}, *ATM*^{wt}*BRCA*^{mt}, and *ATM*^{mt}*BRCA*^{mt}.

In total, 91 lesions were treated in the matched 66 patients where 46 lesions had neither *ATM* or *BRCA* mutations and 45 lesions harbored *ATM* and/or *BRCA* mutations. Nineteen patients had multiple lesions treated and 47 patients had a single lesion treated with RT. Clinical significance of mutations detected in *ATM*, *BRCA1*, and *BRCA2* are summarized in S1 Table. None of the mutations were confirmed to be benign and 47.4%, 63.6%, and 41.9% of mutations were suggested to have at least potential clinical significance in *ATM*, *BRCA1*, and *BRCA2*, respectively. No significant differences in baseline characteristics were observed except for age, primary sites, and RT dose among the *ATM*^{wt}*BRCA*^{wt}, *ATM*^{mt}*BRCA*^{wt}, *ATM*^{wt}*BRCA*^{mt}, and *ATM*^{mt}*BRCA*^{mt} groups (Table 1). RT was delivered in diverse dose schemes, namely conventionally fractionated RT (21 lesions, 23.1%), hypofractionated RT (62 lesions, 68.1%), and stereotactic body radiation therapy (8 lesions, 8.8%). No significant differences in the RT dose scheme were observed among the four groups (Table 1). Most patients received RT to their metastatic lesions. Among the 91 lesions treated, 14 (15.4%) were matched with the sequenced tumor and 38 (41.8%) originated from the metastatic site (Table 1). The frequency of matched RT sites to biopsied sites and the sites of next generation sequencing performed did not significantly differ among the four groups.

2. Treatment outcomes

The median follow-up time was 13.9 months (range, 2.5 to 69.5 months). The treatment response following RT differed significantly among *ATM*^{wt}*BRCA*^{wt}, *ATM*^{mt}*BRCA*^{wt}, *ATM*^{wt}*BRCA*^{mt}, and *ATM*^{mt}*BRCA*^{mt} groups (Fig. 1). A representative *ATM*^{mt}*BRCA*^{mt} case that exhibited CR after RT and the serial change in the diameter of the longest tumor of all lesions are shown in Fig. 1. A majority of patients exhibited reduction in tumor size after RT. Objective response rates were 34.8%, 37.5%, 18.5%, and 80.0% in *ATM*^{wt}*BRCA*^{wt}, *ATM*^{mt}*BRCA*^{wt}, *ATM*^{wt}*BRCA*^{mt}, and *ATM*^{mt}*BRCA*^{mt}, respectively (p=0.007) (S4 Table). Notably, the highest CR rate of 60% was observed in the *ATM*^{mt}*BRCA*^{mt} group, whereas the *ATM*^{wt}*BRCA*^{wt} group showed the lowest CR rate of 2.2% (p < 0.001) (S5 Table). Two lesions with radiological PR and metabolic CR were considered to exhibit CR. Among the 19 patients that had multiple sites treated, 13 (68.4%) exhibited a mixed best tumor response, while six (31.6%) exhibited the same best tumor response. The profound treatment response in the *ATM*^{mt}*BRCA*^{mt} group is also shown in the waterfall plot (Fig. 2A). None of the lesions in the *ATM*^{mt}*BRCA*^{wt} group progressed after RT (Fig. 2A). Mutations in other genes related to DNA damage response pathway, such as *TP53* and *PALB2*, are shown in Fig. 2A, although they did not correlate with response to RT. The median duration of response in patients that had an objective response were 8.7 months (range, 1.4 to 23.9 months), 13.6 months (range, 5.1 to 31.8 months), 4.5 months (range, 1.3 to 14.2 months), and 18.2 months (range, 9.3 to 37.5 months) in the *ATM*^{wt}*BRCA*^{wt}, *ATM*^{mt}*BRCA*^{wt}, *ATM*^{wt}*BRCA*^{mt}, and *ATM*^{mt}*BRCA*^{mt} groups, respectively (Fig. 2B). In addition, all eight lesions (100%) that exhibited an objective response in the *ATM*^{mt}*BRCA*^{mt} group exhibited an ongoing treatment response, while seven out of 16 lesions in the *ATM*^{wt}*BRCA*^{wt} group (43.8%), two out of three lesions in the *ATM*^{mt}*BRCA*^{wt} group (67%), and two out of five lesions in the *ATM*^{wt}*BRCA*^{mt} group (40%) exhibited ongoing response (Fig. 2B). In the univariate analysis, mutation group (p=0.023) was only significantly associated with objective response. Its significance was maintained in the multivariate analysis (p=0.016) (Table 2). The cumulative 1-year LRR in the *ATM*^{wt}*BRCA*^{wt}, *ATM*^{mt}*BRCA*^{wt}, *ATM*^{wt}*BRCA*^{mt}, and *ATM*^{mt}*BRCA*^{mt} groups were 47.9%, 14.3%, 33.2%, and 0%, respectively (p=0.008) (Fig. 3, S4 Table). The differences in cumulative LRR between the *ATM*^{wt}*BRCA*^{wt}, *ATM*^{mt}*BRCA*^{wt}, *ATM*^{wt}*BRCA*^{mt}, and *ATM*^{mt}*BRCA*^{mt} groups tend to be more prominent according to *ATM* mutation status rather

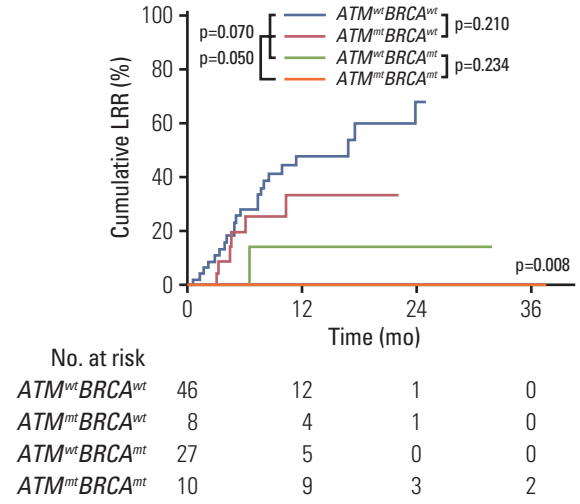

Fig. 3. Local recurrence rate of treated lesions. Shown are the local recurrence rate (LRR) for *ATM*^{wt}*BRCA*^{wt} (n=46), *ATM*^{mt}*BRCA*^{wt} (n=8), *ATM*^{wt}*BRCA*^{mt} (n=27), and *ATM*^{mt}*BRCA*^{mt} (n=10) groups.

Table 3. Treatment-related toxicities after radiotherapy

	$ATM^{wt}BRCA^{wt}$ (n=46)		$ATM^{mt}BRCA^{wt}$ (n=8)		$ATM^{wt}BRCA^{mt}$ (n=27)		$ATM^{mt}BRCA^{mt}$ (n=10)	
	G1-2	G3-4	G1-2	G3-4	G1-2	G3-4	G1-2	G3-4
Skin reaction	0	0	1 (12.5)	0	1 (3.7)	0	0	0
Nausea	5 (10.9)	1 (2.2)	0	0	1 (3.7)	0	1 (10.0)	0
Esophagitis	0	0	0	0	1 (3.7)	0	1 (10.0)	0
Pneumonitis	0	0	0	0	0	0	1 (10.0)	0

Values are presented as number (%).

than *BRCA* mutation status (Fig. 3).

3. Toxicity

The incidence of any treatment-related toxicities after RT was 19.6%, 12.5%, 11.1%, and 20.0%, in the $ATM^{wt}BRCA^{wt}$, $ATM^{mt}BRCA^{wt}$, $ATM^{wt}BRCA^{mt}$, and $ATM^{mt}BRCA^{mt}$ groups, respectively (p=0.792). Most toxicities were limited to grade 1 or 2, and only one event of grade 3 nausea was reported (Table 3). Nausea was most common and occurred in treatments including brain or intra-abdominal lesions. Esophagitis and pneumonitis were reported in treatments including mediastinal lesions.

Discussion

In the present study, we found that tumors harboring mutations in genes related to DNA damage repair exhibit a superior treatment response to RT. Although most previous studies have focused on germline mutations [18,19], we utilized tumor tissues to account for the role of somatic mutations in these tumors. Another strength of this study is that we compared the outcome of RT in patients with mutations to propensity score-matched patients without any mutation. Response to RT varied among patients and depended on factors such as tumor histology, radiation dose, hypoxia, and intrinsic radiosensitivity of tumor cells [3-7]. Among multiple factors, intrinsic cancer cell radiosensitivity is the major factor determining RT response. Considering the mode of action of RT, which induces cell death due to generation of DNA DSBs, the ability of cancer cells to repair DNA damage can considerably affect their radiosensitivity [20].

DNA damage repair involves diverse pathways according to the type of damage induced [8]. DNA DSBs, which are the most common type of DNA damage induced by RT, are repaired via two distinct pathways for repair: the nonhomologous end joining (NHEJ) pathway and the HR pathway The pathway that is activated to repair DNA DSBs depends on the cell cycle phase. NHEJ is dominant in the G1 phase and HR is dominant in the mid-S and mid-G2 phases [21]. Although NHEJ is known to be the major pathway activated to repair DNA DSBs in mammalian cells, HR may also have role in DNA DSB repair [8]. Both BRCA and *ATM* play crucial roles in the repair of DNA DSBs [22,23]. Germline mutation in *ATM* can cause A-T syndrome that is characterized by increased sensitivity to RT [9]. *BRCA1/2* mutation carriers with breast cancer have a higher risk of contralateral breast cancer after breast RT than controls with sporadic breast cancer [10].

BRCA1 and 2 are key molecules mediating the HR pathway, and *BRCA* mutations can disrupt DNA DSB repair [24]. In contrast, compared to BRCA, ATM is involved in the earlier steps of DNA damage repair, as it acts as a sensor of DNA damage [25]. Preclinical studies have demonstrated that pharmacological inhibition of ATM function enhances radiosensitivity [26]. Previously, a case series reported exceptional response to RT in a clinical setting involving eight patients with somatic mutations in *ATM* [27]. We also observed that patients with *ATM* or *BRCA* mutations show better treatment response and lower LRR. Furthermore, patients with *ATM* mutations tended to show better response to RT than those with *BRCA* mutations. ATM not only plays a crucial role in HR but is also involved in NHEJ, the major pathway for DNA DSB repair [28]. As BRCA is mostly involved in HR alone, we suggest that the impact of *ATM* mutations may be more pronounced than *BRCA* mutations in DNA repair dysfunction. *TP53* and *PALB2*, the DNA damage response pathway-related genes included in the next generation sequencing (NGS) panel, did not show clear association with treatment outcome. A previous report also demonstrated lack of association between *TP53* mutation and response to RT [29]. Considering the dominant role of NHEJ in DNA DSB repair, the correlation of somatic alterations in NHEJ pathway genes and response to RT should be investigated in future studies. However, a recent study that analyzed the frequency of somatic alteration in DNA damage repair genes in the Cancer Genome Atlas across 33 cancer types and demonstrated that more HR path-

way genes than NHEJ pathway genes were included in the 50 most frequently mutated DNA damage repair genes [30]. *ATM* was the most commonly mutated gene among DNA DSB repair genes. Due to the lower frequency of somatic mutations in NHEJ pathway genes than HR pathway genes, a larger cohort should be analyzed to properly address the impact of somatic mutations in NHEJ pathway genes on responsiveness to RT.

We also observed that tumors with both *ATM* and *BRCA* mutations have an exceptional response to RT, which was durable. Surprisingly, no local recurrence occurred in lesions with both *ATM* and *BRCA* mutations. Loss-of-function of either *ATM* or *BRCA* alone can lead to activation of alternative pathways to compensate for the dysfunctional DNA damage repair process [21]. However, loss-of-function of both *ATM* and *BRCA* may lead to blockade of both HR and NHEJ pathways, which leads to a more pronounced defect in DNA DSB repair. Considering the exceptional response of ATM^{mt} $BRCA^{mt}$ tumors to RT with a median dose of 45 Gy, we speculated that these tumors may respond well to even lower doses of RT. A previous study demonstrated that *ATM*-deficient cells show defects in DNA DSB repair in an RT dose-independent manner, and that DNA damage persisted even after 0.02 Gy of irradiation [31]. These data indicated the necessity of investigating dose de-escalation while treating $ATM^{mt}BRCA^{mt}$ tumors.

One of the concerns of treating patients with mutations in DNA damage repair-related genes is the higher risk of developing toxicity and secondary malignancy [19,32]. The chances of developing toxicity and secondary malignancy are low as long as the mutation is not present in the germline, as normal cells do not harbor such mutations and have intact DNA damage repair pathways. In our study, we did not observe any significant increase in toxicity after irradiating tumors with *ATM* or *BRCA* mutations, which implies that RT dose constraints similar to those used for normal organs may be used in these patients compared to those without mutations.

This study had several limitations which made the results less conclusive. The sample size was small due to the limited number of patients who received both RT and underwent NGS. Characteristics of the patients were also heterogeneous regarding RT dose and primary site. In addition, we were only able to match patients with or without mutations rather than the four subgroups due to the limitation in the number of patients, which resulted in significant differences in age and primary site among the subgroups. Furthermore, we were not able to analyze the effect of mutations according to tumor histology or different RT doses. Further analysis in a larger cohort of patients is required to confirm our results. The follow-up period was relatively short to account for late RT-related toxicities and secondary malignancies. Furthermore, sequencing was only performed in tumor tissue and we were not able to properly filter germline variants. However, variant allele frequency (S6 Fig.) suggested that most mutations are possibly somatic instead of germline. Among DNA damage repair genes, mutations in only *ATM* and *BRCA* were evaluated, as other HR and NHEJ pathway-related genes were not included in the NGS panel. For competing risk analysis of multiple lesions treated in the same individual, a stratified analysis that adjusts for the effect of each individual should be performed. However, in the present study, this could not be performed due to the lack of local recurrence events in the $ATM^{mt}BRCA^{mt}$ group [33]. Moreover, multivariate analysis of LRR could not be performed due to the lack of local recurrence events in the $ATM^{mt}BRCA^{mt}$ group.

In this hypothesis generating study, we found that tumors with *ATM* or *BRCA* mutations may have enhanced radiosensitivity, and that the presence of both mutations may lead to exceptional response to RT. In the era of personalized medicine, continuous efforts are being made to customize RT dose according to the intrinsic radiosensitivity of the tumor [7,34]. Taking advantage of NGS, we can now easily evaluate the patients' mutation profile to identify radiosensitizing mutations, such as in *ATM* and *BRCA* [35]. Further investigations are required to confirm whether such mutations confer radiosensitivity and whether de-escalation of RT dose is feasible for tumors harboring such mutations. In addition, future studies can focus on using combinations of DNA repair inhibitors. As DNA repair inhibitors have entered clinical trials as single agents and as image-guided, intensity-modulated radiation therapy has been successful in decreasing RT toxicity, the onus lies on the radiation oncology community to carefully design clinical trials.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (https://www.e-crt.org).

Ethical Statement

This retrospective study was approved by the Institutional Review Board of the Severance Hospital, Seoul, Korea, which also waived the need to obtain informed consent because data were analyzed anonymously.

Author Contributions

Conceived and designed the analysis: Kim KH, Kim HS, Yoon HI, Ahn JB, Chang JS.

Collected the data: Kim KH, Kim HS, Kim SS, Shim HS, Yang AJ, Lee JJB, Chang JS.

Contributed data or analysis tools: Kim KH, Kim HS, Kim SS, Shim HS, Yang AJ, Lee JJB, Yoon HI, Ahn JB, Chang JS.

Performed the analysis: Kim KH, Kim HS, Yang AJ, Lee JJB, Chang

JS.
Wrote the paper: Kim KH, Kim HS, Yang AJ, Lee JJB, Chang JS.

Conflicts of Interest
Conflict of interest relevant to this article was not reported.

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