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High *SLC20A1* Expression Is Associated With Poor Prognosis for Radiotherapy of Estrogen Receptor-positive Breast Cancer

CHOTARO ONAGA[1], SHOMA TAMORI[1], IZUMI MATSUOKA[1], AYAKA OZAKI[1], HITOMI MOTOMURA[1], YUKA NAGASHIMA[1], TSUGUMICHI SATO[2], KEIKO SATO[3], KOUJI TAHATA[3], YUYUN XIONG[1], YOSHIO NAKANO[2], YASUNARI MANO[2], SATORU MIYAZAKI[2], KAZUNORI SASAKI[4], SHIGEO OHNO[4] and KAZUNORI AKIMOTO[1]

[1]*Department of Medicinal and Life Sciences, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba, Japan;*
[2]*Department of Pharmacy, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba, Japan;*
[3]*Department of Information Sciences, Faculty of Science and Technology, Tokyo University of Science, Chiba, Japan;*
[4]*Laboratory of Cancer Biology, Institute for Diseases of Old Age, Juntendo University School of Medicine, Tokyo, Japan*

Abstract. *Background/Aim: Radiotherapy is one of the main treatments for estrogen receptor-positive (ER+) breast cancer. However, in some ER+ breast cancer cases, radiotherapy is insufficient to inhibit progression and there is a lack of markers to predict radiotherapy insensitivity. Solute carrier family 20 member 1 (SLC20A1) is a sodium/inorganic phosphate symporter, which has been proposed to be a viable prognostic marker for luminal A and B types of ER+ breast cancer. The present study examined the possibility of SLC20A1 as a novel biomarker for the prediction of radiotherapy efficiency. Patients and Methods: The Molecular Taxonomy of Breast Cancer International Consortium dataset was downloaded from cBioportal and the prognosis of patients with high SLC20A1 expression (SLC20A1^{high}) was compared with that of patients with low SLC20A1 expression, without or with radiotherapy and tumor stages I, II, and III, using the Kaplan–Meier method and multivariate Cox regression analyses of disease-specific and relapse-free survival. Results: Patients in the SLC20A1^{high} group with radiotherapy showed poor clinical outcomes in both luminal A and luminal B breast cancers. Furthermore, in luminal A breast cancer at tumor stage I, patients in the SLC20A1^{high} group with radiotherapy also showed poor clinical outcomes. Therefore, these results suggest that radiotherapy is insufficient for patients in the SLC20A1^{high} group for both luminal A and B types, and especially for the luminal A type at tumor stage I. Conclusion: SLC20A1 can be used as a prognostic marker for the prediction of the efficacy of radiotherapy for luminal A and luminal B breast cancers.*

Correspondence to: Kazunori Akimoto, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan. Tel: +81 471213614, Fax: +81 471213614, e-mail: akimoto@rs.tus.ac.jp

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Breast cancer is the most common cancer in women worldwide (1). Breast cancer treatment is decided according to the subtype and tumor stage (2-4). Estrogen receptor-positive (ER+) breast cancer is a major subtype of breast cancer that accounts for 70-80% of breast cancer cases and is associated with a good prognosis compared with other subtypes (3, 5, 6). Radiotherapy is one of the main treatments for solid cancer types, including breast cancer. However, in some patients with cancer, radiotherapy may cause tumor repopulation (7, 8). In breast cancer, radiotherapy by X-ray suppresses first recurrence and cancer death; however, there are still patients who relapse and succumb to cancer (9). Breast cancer is stratified into at least six subtypes based on the gene expression pattern [Prediction Analysis of Microarray 50 (PAM50)]: Normal-like, luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-enriched, claudin-low and basal-like (10-14). Both luminal A and luminal B types are ER+ breast cancer types

and are mainly treated by surgery, radiotherapy, and endocrine therapy (5, 6). However, radiation sensitivities are not associated with the respective intrinsic subtypes of breast cancer (15). Therefore, it is necessary to identify novel biomarkers to stratify breast cancer in detail and to predict the efficiency of radiotherapy.

Solute carrier family 20 member 1 (SLC20A1) is a gene that encodes a sodium/phosphate symporter (16, 17). In HeLa cervical cancer cells and HepG2 hepatocellular carcinoma cells, SLC20A1 knockdown induces the suppression of cancer cell proliferation (18). Our previous data showed that higher *SLC20A1* expression indicates poor prognosis in patients with breast cancer subtypes such as the luminal A and B, claudin-low, and basal-like types (19, 20). Furthermore, higher *SLC20A1* expression also indicates poor clinical outcomes for patients with claudin-low and basal-like breast cancer treated with radiotherapy (20). However, the association between *SLC20A1* gene expression and the effect of radiotherapy in ER+ breast cancer remains to be determined.

The present study demonstrated that patients with luminal A or luminal B breast cancer in the high SLC20A1 expression (*SLC20A1*^{high}) group who were subjected to radiotherapy showed poor clinical outcomes. Furthermore, among patients with luminal A breast cancer at tumor stage I, patients in the *SLC20A1*^{high} group showed poor clinical outcomes after radiotherapy. Therefore, these results suggest that radiotherapy for patients in the *SLC20A1*^{high} group is insufficient for both luminal A and luminal B breast cancer, particularly for the luminal A type at tumor stage I.

Patients and Methods

Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) dataset. The METABRIC dataset (21, 22) was downloaded from cBioportal (http://cbioportal.org) (23, 24) on July 29, 2020. The clinicopathological data of these patients have been reported previously (20, 25, 26). The average values of the ages at diagnosis in the entire cohort and luminal A and luminal B are as follows [all: 61.09 years (21.93-96.29), luminal A: 62.78 years (26.36-90.23), luminal B: 65.21 years (28.62-92.14)]. This METABRIC dataset contains mRNA expression profile data (luminal A: n=679, luminal B: n=461). The optimal cut-off thresholds were defined using Youden's index to assign the patients into the *SLC20A1*^{high} and low SLC20A1 expression (*SLC20A1*^{low}) groups through receiver operating characteristic (ROC) analysis. ROC analysis was performed for SLC20A1 gene expression data and disease-specific survival (DSS) or relapse-free status (RFS) for each group divided by tumor-stage and radiotherapy, and Youden's index was calculated. Patient vital status data of 'living' and 'died of disease' were used for DSS and relapse-free status was used for RFS. The patients without or with radiotherapy were divided according to YES and NO in the items of radiotherapy in clinical data. The number of radiotherapies in luminal A and luminal B are as follows (radiotherapy; luminal A: NO, n=312, YES, n=367, luminal B: NO, n=171, YES, n=290).

Analysis of patient prognosis using the Kaplan–Meier method. Survival curves based on DSS and RFS were plotted using the Kaplan–Meier method. The curves were compared between the *SLC20A1*^{high} and *SLC20A1*^{low} groups using the log-rank (Cochran-Mantel-Haenszel) test. Kaplan–Meier survival curves were generated using BellCurve for Excel version 3.00 (Social Survey Research Information, Tokyo, Japan).

Analysis of patient prognosis using the multivariate Cox regression method. Multivariate Cox regression analysis was performed to evaluate the influence of high and low SLC20A1 gene expression on patient outcome and to estimate the adjusted hazard ratios (HRs) of the *SLC20A1*^{high} group relative to the *SLC20A1*^{low} group for DSS or RFS. Diagnosis age, endocrine therapy and chemotherapy were set as confounding factors to remove their effect. The number of endocrine therapy and chemotherapy in luminal A and luminal B are as follows (endocrine therapy; luminal A: NO, n=218, YES, n=461, luminal B: NO, n=92, YES, n=369) (chemotherapy; luminal A: NO, n=625, YES, n=54, luminal B: NO, n=416, YES, n=45). The level of significance was set at 5% (two-sided). Multivariate Cox regression analyses were carried out using BellCurve for Excel version 3.00 (Social Survey Research Information).

Analysis of the recurrence incidence rate. The recurrence incidence rate where the number of recurrences divided by the observation period of the patients with luminal A and luminal B breast cancer with radiotherapy was calculated. The observation period was defined as relapse-free survival time. Follow-up was censored if a patient had a relapse, died, or dropped out due to any other reasons. The observation period was divided every 5 years, where the number of recurrences was then counted during that term. The *p*-value was calculated from the statistical analysis based on normal distribution and corrected using the Holm method. The incidence rate ratio was calculated as the ratio of the recurrence incidence rate of the *SLC20A1*^{high} group to that of the *SLC20A1*^{low} group.

Results

Kaplan–Meier analysis indicates that radiotherapy is insufficient for SLC20A1^{high} *luminal A and luminal B breast cancer.* To examine the effect of radiotherapy on *SLC20A1*^{high} luminal A and luminal B tumors, Kaplan–Meier analysis of DSS and RFS was performed. For the luminal A type, patients in the *SLC20A1*^{high} group both without and with radiotherapy showed poor clinical outcomes compared with *SLC20A1*^{low} group (without radiotherapy; DSS, $p<0.001$, RFS, $p=0.0024$, with radiotherapy; DSS, $p=0.0032$, RFS, $p=0.0095$) (Figure 1A-D). For the luminal B type, although patients in the *SLC20A1*^{high} group without radiotherapy showed good clinical outcomes compared with *SLC20A1*^{low} group ($p=0.045$) (Figure 1E), patients in the *SLC20A1*^{high} group with radiotherapy showed poor clinical outcomes compared with *SLC20A1*^{low} group ($p=0.041$) (Figure 1F). Furthermore, regarding RFS of patients with luminal B breast cancer, there was no significant difference between patients in the *SLC20A1*^{high} and *SLC20A1*^{low} groups without radiotherapy ($p=0.056$) (Figure 1G) but patients in the *SLC20A1*^{high} group with radiotherapy had a short interval

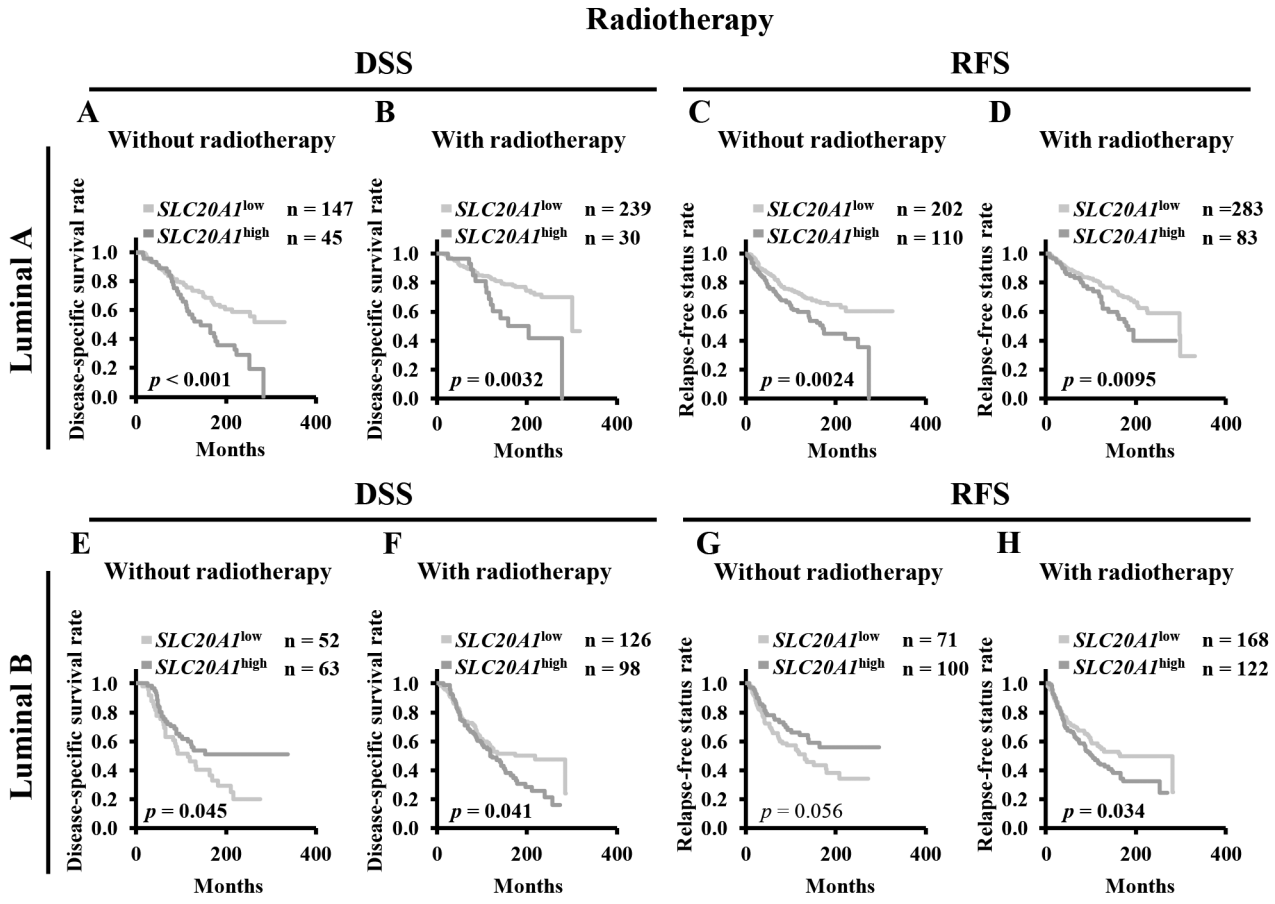


Figure 1. *High solute carrier family 20 member 1 (SLC20A1) expression (SLC20A1^{high}) is associated with poorer clinical outcomes in the luminal A and luminal B breast cancer subtypes following radiotherapy. (A-D) Kaplan–Meier analyses comparing disease-specific survival (DSS) and relapse-free status (RFS) between the SLC20A1^{high} and low SLC20A1 expression (SLC20A1^{low}) groups of patients with luminal A breast cancer without or with radiotherapy. (A) DSS in the group without radiotherapy. (B) DSS in the group with radiotherapy. (C) RFS in the group without radiotherapy. (D) RFS in the group with radiotherapy. (E-H) Kaplan–Meier analyses comparing DSS and RFS between the SLC20A1^{high} and SLC20A1^{low} groups of patients with luminal B breast cancer without or with radiotherapy. (E) DSS in the group without radiotherapy. (F) DSS in the group with radiotherapy. (G) RFS in the group without radiotherapy. (H) RFS in the group with radiotherapy.*

until recurrence compared with *SLC20A1*^{low} group (p=0.034) (Figure 1H). These results suggest that radiotherapy is insufficient for patients in the *SLC20A1*^{high} group with luminal A and luminal B breast cancer.

Multivariate Cox regression analysis also indicates that radiotherapy is insufficient for patients in the SLC20A1^{high} group with luminal A and luminal B breast cancer. Multivariate Cox regression analysis of DSS with age as a confounding factor was subsequently performed. Patients in the *SLC20A1*^{high} group with luminal A breast cancer both without and with radiotherapy showed poor clinical outcomes compared with *SLC20A1*^{low} group [without radiotherapy; HR=1.83, 95% confidence interval (95%CI)=1.16-2.89, with radiotherapy; HR=2.37, 95%CI=1.31-4.30] (Table I). On the other hand, in patients with luminal B breast cancer both without and with radiotherapy, there was no statistically significant difference regarding clinical outcomes between the *SLC20A1*^{high} and *SLC20A1*^{low} groups (without radiotherapy; HR=0.64, 95%CI=0.38-1.05, with radiotherapy; HR=1.41, 95%CI=0.99-2.03) (Table I). In multivariate Cox regression analysis of DSS with age, endocrine therapy, and chemotherapy as confounding factors, *SLC20A1*^{high} was also associated with poor clinical outcome for luminal A breast cancer compared with *SLC20A1*^{low} (without radiotherapy; HR=1.93, 95%CI=1.21-3.06, with radiotherapy; HR=2.71, 95%CI=1.48-4.94) (Table I). For luminal B breast cancer, both without and with radiotherapy, there was no statistically significant difference regarding clinical outcomes between the patients in the *SLC20A1*^{high} and *SLC20A1*^{low} groups (without

Table I. *Multivariate Cox regression analyses of disease-specific survival (DSS) and relapse-free status (RFS) in patients with luminal A and luminal B breast cancer without or with radiotherapy.*

	DSS		
Cofounding factor: age	Hazard ratio[a]	95% Confidence interval	*p*-Value
Without radiotherapy			
Luminal A	**1.83[a]**	**1.16-2.89**	**0.010**
Luminal B	0.64[a]	0.38-1.06	0.083
With radiotherapy			
Luminal A	**2.37[a]**	**1.31-4.30**	**0.0044**
Luminal B	1.41[a]	0.99-2.03	0.060
Cofounding factor: age, endocrine-, chemo-therapy	Hazard ratio[b]	95% Confidence interval	*p*-Value
Without radiotherapy			
Luminal A	**1.93[b]**	**1.21-3.06**	**0.0055**
Luminal B	0.75[b]	0.44-1.27	0.28
With radiotherapy			
Luminal A	**2.71[b]**	**1.48-4.94**	**0.0012**
Luminal B	1.37[b]	0.95-1.97	0.089
	RFS		
Cofounding factor: age	Hazard ratio[a]	95% Confidence interval	*p*-Value
Without radiotherapy			
Luminal A	**1.76[a]**	**1.22-2.55**	**0.0027**
Luminal B	0.62[a]	0.38-1.01	0.054
With radiotherapy			
Luminal A	**1.71[a]**	**1.14-2.58**	**0.010**
Luminal B	**1.42[a]**	**1.02-1.98**	**0.036**
Cofounding factor: age, endocrine-, chemo-therapy	Hazard ratio[b]	95% Confidence interval	*p*-Value
Without radiotherapy			
Luminal A	**1.76[b]**	**1.21-2.55**	**0.0030**
Luminal B	0.63[b]	0.39-1.02	0.063
With radiotherapy			
Luminal A	**1.67[b]**	**1.11-2.52**	**0.014**
Luminal B	**1.43[b]**	**1.02-1.99**	**0.037**

Hazard ratio: hazard ratio of DSS or RFS for the *SLC20A1*^{high} group compared with *SLC20A1*^{low} group adjusted by [a]age only, or [b]age, endocrine therapy, and chemotherapy using Cox proportional hazard model. Significant differences are shown in bold.

radiotherapy; HR=0.75, 95%CI=0.44-1.27, with radiotherapy; HR=1.37, 95%CI=0.95-1.97) (Table I). Multivariate Cox regression analyses of RFS with age as a confounding factor indicated that patients in the *SLC20A1*^{high} group with luminal A breast cancer with radiotherapy had a short interval until recurrence compared with *SLC20A1*^{low} group (without radiotherapy; HR=1.76, 95%CI=1.22-2.55, with radiotherapy; HR=1.71, 95%CI=1.14-2.58) (Table I). Unlike for DSS, multivariate Cox regression analyses of RFS with age as a confounding factor indicated that patients in the *SLC20A1*^{high} group with luminal B breast cancer with radiotherapy had a short interval until recurrence compared with *SLC20A1*^{low} group (without radiotherapy; HR=0.62, 95%CI=0.38-1.01, with radiotherapy; HR=1.42, 95%CI=1.02-1.98). The analyses with age, endocrine therapy and chemotherapy as a confounding factor indicated poor clinical outcome for *SLC20A1*^{high} patients with luminal A breast cancer compared with *SLC20A1*^{low} patients (without radiotherapy; HR=1.76, 95%CI=1.21-2.55, with radiotherapy; HR=1.67, 95%CI=1.11-2.52). In luminal B breast cancer, although patients in the *SLC20A1*^{high} group without radiotherapy did not show poor outcomes (HR=0.63, 95%CI=0.39-1.02), patients in the *SLC20A1*^{high} group with radiotherapy showed poor clinical outcomes compared with those in the *SLC20A1*^{low} group (HR=1.43, 95%CI=1.02-1.99) (Table I). These results strongly suggest that radiotherapy is insufficient for patients

in the *SLC20A1*^{high} group with ER+ breast cancer, such as luminal A and luminal B breast cancer.

Kaplan–Meier analyses indicate that radiotherapy is insufficient for patients in the SLC20A1^{high} group with luminal A breast cancer at tumor stage I. To examine the effect of radiotherapy at each tumor stage, Kaplan–Meier analysis comparing DSS between the *SLC20A1*^{high} and *SLC20A1*^{low} groups was subsequently performed for patients with luminal A and luminal B breast cancer. At tumor stage I, patients in the *SLC20A1*^{high} group with luminal A breast cancer both without and with radiotherapy showed poor clinical outcomes compared with those in the *SLC20A1*^{low} group (without radiotherapy; $p<0.001$, with radiotherapy; $p<0.001$) (Figure 2A and B). At tumor stage II, patients in the *SLC20A1*^{high} group with luminal A breast cancer without radiotherapy showed poor clinical outcomes, but patients in the *SLC20A1*^{high} group with luminal A breast cancer with radiotherapy did not show poor clinical outcomes compared with *SLC20A1*^{low} group (without radiotherapy; $p=0.0038$, with radiotherapy; $p=0.15$) (Figure 2E and F). At tumor stage III, Kaplan–Meier analysis did not reveal a significant difference between patients in the *SLC20A1*^{high} and *SLC20A1*^{low} groups with luminal A breast cancer both without and with radiotherapy (without radiotherapy; $p=0.23$, with radiotherapy; $p=0.050$) (Figure 2I and J).

The present study subsequently examined the association between RFS and radiotherapy *via* Kaplan–Meier analysis. At tumor stage I, patients in the *SLC20A1*^{high} group with luminal A breast cancer both without and with radiotherapy showed a short interval until recurrence compared with those in the *SLC20A1*^{low} group (without radiotherapy; $p=0.032$, with radiotherapy; $p<0.001$) (Figure 2C and D). Unlike for tumor stage I, in luminal A breast cancer at tumor stage II, although patients in the *SLC20A1*^{high} group without radiotherapy showed a short interval until recurrence, patients in the *SLC20A1*^{high} group with radiotherapy did not show a short interval until recurrence compared with those in the *SLC20A1*^{low} group (without radiotherapy; $p=0.0045$, with radiotherapy; $p=0.14$) (Figure 2G and H). At tumor stage III, patients with luminal A breast cancer in the *SLC20A1*^{high} group both without and with radiotherapy did not show a significant difference compared with patients in the *SLC20A1*^{low} group (without radiotherapy; $p=0.18$, with radiotherapy; $p=0.72$) (Figure 2K and L).

In luminal B breast cancer at tumor stages I and II, patients in the *SLC20A1*^{high} group both without and with radiotherapy did not show poor clinical outcomes compared with patients in the *SLC20A1*^{low} group (tumor stage I; without radiotherapy; $p=0.13$, with radiotherapy; $p=0.18$, tumor stage II; without radiotherapy; $p=0.074$, with radiotherapy; $p=0.20$) (Figure 3A, B, E and F). At tumor stage III, the number of patients with luminal B breast cancer without radiotherapy was not sufficient for analysis. Patients in the *SLC20A1*^{high} group with luminal B breast cancer with radiotherapy at tumor stage III did not exhibit a significant difference compared with those in the *SLC20A1*^{low} group [without radiotherapy; not determined (N.D.), with radiotherapy; $p=0.80$] (Figure 3I and J).

At all tumor stages from I to III, patients in the *SLC20A1*^{high} group with luminal B breast cancer without and with radiotherapy did not exhibit a significant difference compared with patients in the *SLC20A1*^{low} group (tumor stage I: without radiotherapy; $p=0.17$, with radiotherapy; $p=0.23$, tumor stage II: without radiotherapy; $p=0.14$, with radiotherapy; $p=0.061$, tumor stage III: without radiotherapy; $p=0.50$, with radiotherapy; $p=0.20$) (Figure 3C-D, G-H and K-L).

Multivariate Cox regression analysis also indicates that radiotherapy is insufficient for patients in the SLC20A1^{high} group with luminal A breast cancer at tumor stage I. Multivariate Cox regression analysis for DSS and RFS with age as a confounding factor was subsequently performed. At tumor stage I, patients with luminal A breast cancer in the *SLC20A1*^{high} group both without and with radiotherapy showed poor clinical outcomes compared with those in the *SLC20A1*^{low} group (DSS: without radiotherapy; HR=4.54, 95%CI=1.76-11.69, with radiotherapy; HR=7.60, 95%CI=2.82-20.51, RFS: without radiotherapy; HR=2.22, 95%CI=1.05-4.68, with radiotherapy; HR=3.66, 95%CI=1.79-7.49) (Table II). At tumor stage II, patients with luminal A breast cancer in the *SLC20A1*^{high} group without radiotherapy showed poor clinical outcomes; however, patients with luminal A breast cancer in the *SLC20A1*^{high} group with radiotherapy did not show poor clinical outcomes compared with those in the *SLC20A1*^{low} group (DSS: without radiotherapy; HR=2.74, 95%CI=1.24-6.05, with radiotherapy; HR=1.63, 95%CI=0.80-3.33, RFS: without radiotherapy; HR=2.74, 95%CI=1.32-5.67, with radiotherapy; HR=1.61, 95%CI=0.86-3.03) (Table II). At tumor stage III, there were too few patients with luminal A breast cancer without radiotherapy to perform Cox analyses. There were no significant differences between the *SLC20A1*^{high} and *SLC20A1*^{low} groups of patients with luminal A breast cancer with radiotherapy (DSS: without radiotherapy; N.D., with radiotherapy; HR=2.12, 95%CI=0.48-9.39, RFS: without radiotherapy; N.D., with radiotherapy; HR=1.23, 95%CI=0.36-4.13) (Table II). For luminal B breast cancer, there were no significant differences between the *SLC20A1*^{high} and *SLC20A1*^{low} groups of patients at all tumor stages from tumor stage I to III (tumor stage I, DSS: without radiotherapy; HR=4.63, 95%CI=0.56-38.36, with radiotherapy; HR=1.88, 95%CI=0.73-4.80, RFS: without radiotherapy; HR=2.62, 95%CI=0.58-11.74, with radiotherapy; HR=1.74, 95%CI=0.77-3.94, tumor stage II, DSS: without radiotherapy; HR=0.58, 95%CI=0.23-1.46, with radiotherapy; HR=1.33, 95%CI=0.80-2.19, RFS:

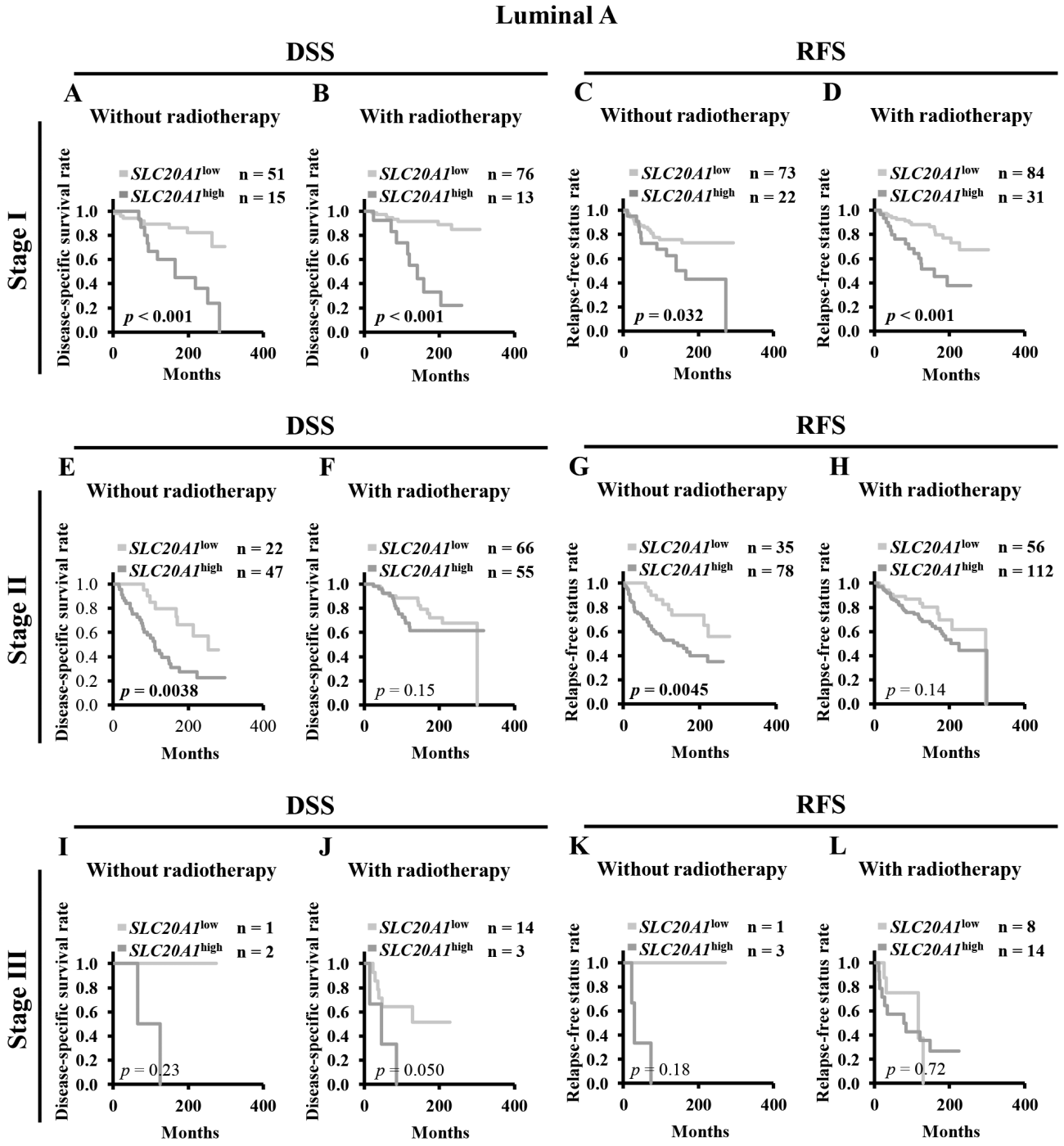


Figure 2. *High solute carrier family 20 member 1 (SLC20A1) expression ($SLC20A1^{high}$) is associated with poorer clinical outcomes in patients with luminal A breast cancer at tumor stage I with radiotherapy. (A-D) Kaplan–Meier analyses comparing disease-specific survival (DSS) and relapse-free status (RFS) between the $SLC20A1^{high}$ and low SLC20A1 expression ($SLC20A1^{low}$) groups of patients with stage I luminal A breast cancer without or with radiotherapy. (A) DSS in the group without radiotherapy. (B) DSS in the group with radiotherapy. (C) RFS in the group without radiotherapy. (D) RFS in the group with radiotherapy. (E-H) Kaplan–Meier analyses comparing DSS and RFS between the $SLC20A1^{high}$ and $SLC20A1^{low}$ groups of patients with stage II luminal A breast cancer without or with radiotherapy. (E) DSS in the group without radiotherapy. (F) DSS in the group with radiotherapy. (G) RFS in the group without radiotherapy. (H) RFS in the group with radiotherapy. (I-L) Kaplan–Meier analyses comparing DSS and RFS between the $SLC20A1^{high}$ and $SLC20A1^{low}$ groups of patients with stage III luminal A breast cancer without or with radiotherapy. (I) DSS in the group without radiotherapy. (J) DSS in the group with radiotherapy. (K) RFS in the group without radiotherapy. (L) RFS in the group with radiotherapy.*

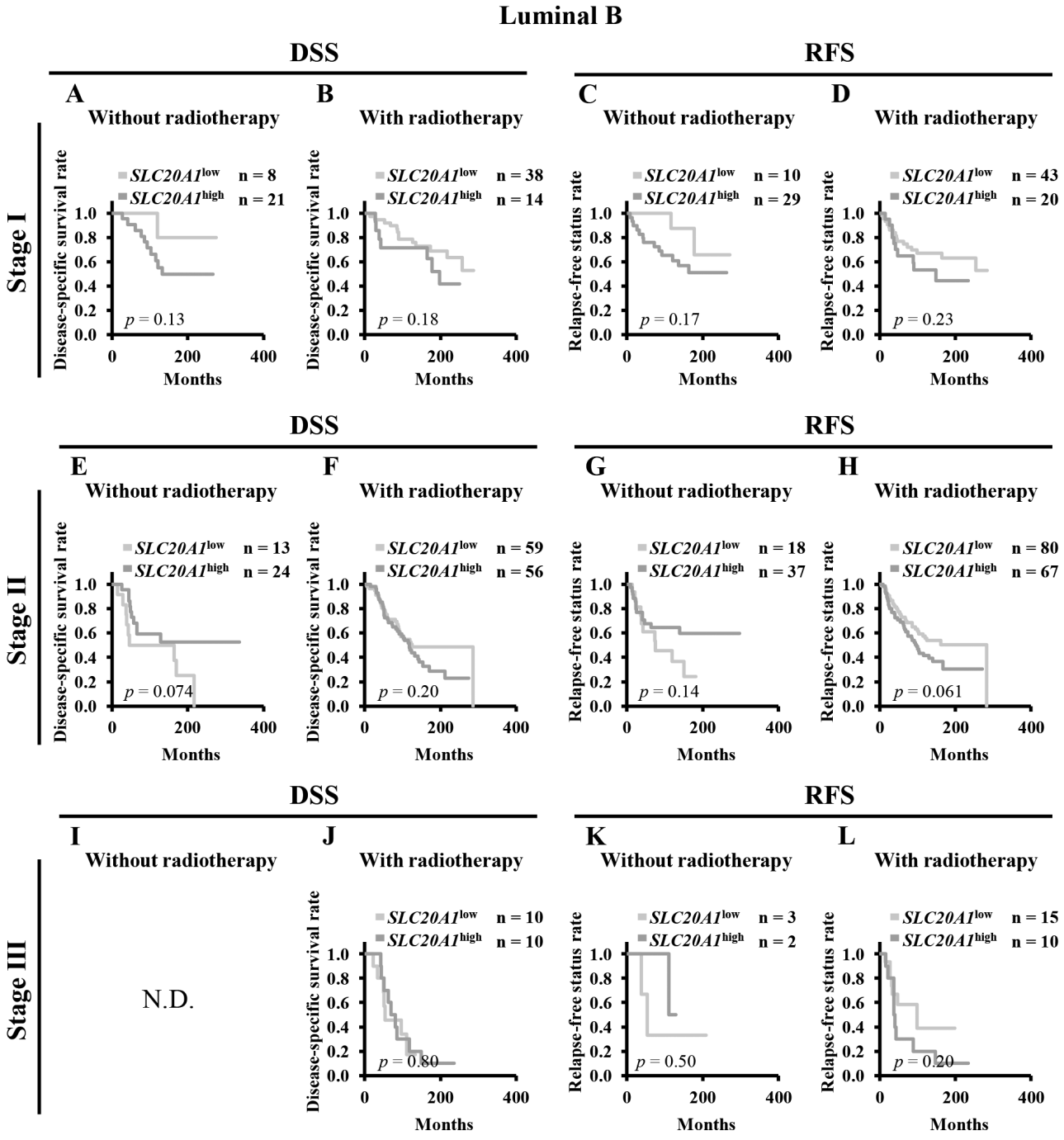


Figure 3. *There are no significant differences between high solute carrier family 20 member 1 (SLC20A1) expression (SLC20A1^{high}) and low SLC20A1 expression (SLC20A1^{low}) in patients with luminal breast cancer undergoing radiotherapy. (A-D) Kaplan–Meier analyses comparing disease-specific survival (DSS) and relapse-free status (RFS) between the SLC20A1^{high} and SLC20A1^{low} groups of patients with stage I luminal B breast cancer without or with radiotherapy. (A) DSS in the group without radiotherapy. (B) DSS in the group with radiotherapy. (C) RFS in the group without radiotherapy. (D) RFS in the group with radiotherapy. (E-H) Kaplan–Meier analyses comparing DSS and RFS between the SLC20A1^{high} and SLC20A1^{low} groups of patients with stage II luminal B breast cancer without or with radiotherapy. (E) DSS in the group without radiotherapy. (F) DSS in the group with radiotherapy. (G) RFS in the group without radiotherapy. (H) RFS in the group with radiotherapy. (I-L) Kaplan–Meier analyses comparing DSS and RFS between the SLC20A1^{high} and SLC20A1^{low} groups of patients with stage III luminal B breast cancer without or with radiotherapy. (I) DSS in the group without radiotherapy. (J) DSS in the group with radiotherapy. (K) RFS in the group without radiotherapy. (L) RFS in the group with radiotherapy. (I) There was an insufficient number of patients for the Kaplan–Meier analysis. N.D. stands for "not determined".*

Table II. *Multivariate Cox regression analyses of disease-specific survival (DSS) and relapse-free status (RFS) in patients with luminal A and luminal B breast cancer without or with radiotherapy at tumor-stage I, II and III.*

	DSS		
Cofounding factor: age	Hazard ratio[a]	95% Confidence interval	*p*-Value
Stage I			
Without radiotherapy			
Luminal A	**4.54[a]**	**1.76-11.69**	**0.017**
Luminal B	4.63[a]	0.56-38.36	0.16
With radiotherapy			
Luminal A	**7.60[a]**	**2.82-20.51**	**<0.001**
Luminal B	1.88[a]	0.73- 4.80	0.19
Stage II			
Without radiotherapy			
Luminal A	**2.74[a]**	**1.24-6.05**	**0.013**
Luminal B	0.58[a]	0.23-1.46	0.25
With radiotherapy			
Luminal A	1.63[a]	0.80-3.33	0.18
Luminal B	1.33[a]	0.80-2.19	0.27
Stage III			
Without radiotherapy			
Luminal A	N.D.[a]	N.D.	N.D.
Luminal B	N.D.[a]	N.D.	N.D.
With radiotherapy			
Luminal A	2.12[a]	0.48-9.39	0.32
Luminal B	0.87[a]	0.30-2.50	0.79
Cofounding factor: age, endocrine-, chemo-therapy	Hazard ratio[b]	95% Confidence interval	*p*-Value
Stage I			
Without radiotherapy			
Luminal A	N.D.[b]	N.D.	N.D.
Luminal B	N.D.[b]	N.D.	N.D.
With radiotherapy			
Luminal A	N.D.b	N.D.	N.D.
Luminal B	N.D.b	N.D.	N.D.
Stage II			
Without radiotherapy			
Luminal A	**2.99[b]**	**1.34-6.71**	**0.0077**
Luminal B	0.75[b]	0.23-2.38	0.62
With radiotherapy			
Luminal A	1.62[b]	0.79-3.31	0.19
Luminal B	1.31[b]	0.79-2.18	0.29
Stage III			
Without radiotherapy			
Luminal A	N.D.[b]	N.D.	N.D.
Luminal B	N.D.[b]	N.D.	N.D.
With radiotherapy			
Luminal A	1.85[b]	0.32-10.61	0.49
Luminal B	0.65[b]	0.19-2.19	0.48
	RFS		
Cofounding factor: age	Hazard ratio[a]	95% Confidence interval	*p*-Value
Stage I			
Without radiotherapy			
Luminal A	**2.22[a]**	**1.05-4.68**	**0.037**
Luminal B	2.62[a]	0.58-11.74	0.21

Table II. *Continued*

Table II. *Continued*

	RFS		
Cofounding factor: age	Hazard ratio[a]	95% Confidence interval	*p*-Value
With radiotherapy			
Luminal A	**3.66[a]**	**1.79-7.49**	**<0.001**
Luminal B	1.74[a]	0.77-3.94	0.18
Stage II			
Without radiotherapy			
Luminal A	**2.74[a]**	**1.32-5.67**	**0.0066**
Luminal B	0.53[a]	0.23-1.22	0.14
With radiotherapy			
Luminal A	1.61[a]	0.86-3.03	0.14
Luminal B	1.53[a]	0.96-2.44	0.077
Stage III			
Without radiotherapy			
Luminal A	N.D.[a]	N.D.	N.D.
Luminal B	0.43[a]	0.036-5.25	0.51
With radiotherapy			
Luminal A	1.23[a]	0.36-4.13	0.74
Luminal B	1.92[a]	0.70-5.29	0.21
	RFS		
Cofounding factor: age, endocrine-, chemo-therapy	Hazard ratio[b]	95% Confidence interval	*p*-Value
Stage I			
Without radiotherapy			
Luminal A	N.D.[b]	N.D.	N.D.
Luminal B	N.D.[b]	N.D.	N.D.
With radiotherapy			
Luminal A	N.D.[b]	N.D.	N.D.
Luminal B	N.D.[b]	N.D.	N.D.
Stage II			
Without radiotherapy			
Luminal A	**2.79[b]**	**1.35-5.79**	**0.0058**
Luminal B	0.67[b]	0.25-1.80	0.43
With radiotherapy			
Luminal A	1.71[b]	0.91-3.24	0.097
Luminal B	1.53[b]	0.95-2.47	0.082
Stage III			
Without radiotherapy			
Luminal A	N.D.[b]	N.D.	N.D.
Luminal B	N.D.[b]	N.D.	N.D.
With radiotherapy			
Luminal A	1.85[b]	0.54-6.35	0.32
Luminal B	2.31[b]	0.63-8.43	0.21

Hazard ratio: hazard ratio of DSS or RFS for the *SLC20A1*^{high} group compared with *SLC20A1*^{low} group adjusted by [a]age only, or [b]age, endocrine therapy, and chemotherapy using Cox proportional hazard model. Significant differences are shown in bold.

without radiotherapy; HR=0.53, 95%CI=0.23-1.22, with radiotherapy; HR=1.53, 95%CI=0.96-2.44, tumor stage III, DSS: without radiotherapy; N.D., with radiotherapy; HR=0.87, 95%CI=0.30-2.50, RFS: without radiotherapy; HR=0.43, 95%CI=0.036-5.25, with radiotherapy; HR=1.92, 95%CI=0.70-5.29) (Table II). On the other hand, there were too few patients with luminal A and luminal B breast cancer at tumor stage I and III to perform multivariate Cox regression analyses of DSS and RFS with age, endocrine therapy, and chemotherapy as confounding factors. At tumor stage II, although patients with luminal A breast cancer in the *SLC20A1*^{high} group without radiotherapy showed poor

clinical outcomes and had a short interval until recurrence, patients with luminal A breast cancer in the *SLC20A1*^{high} group with radiotherapy did not show poor clinical outcomes and a short interval until recurrence compared with those in the *SLC20A1*^{low} group. In patients with luminal B breast cancer, there was no significant difference between the *SLC20A1*^{high} and *SLC20A1*^{low} groups of patients both without and with radiotherapy. Therefore, consistent with the results of Kaplan–Meier analyses, multivariate Cox regression analyses indicated that radiotherapy for patients with luminal A breast cancer at tumor stage I is insufficient.

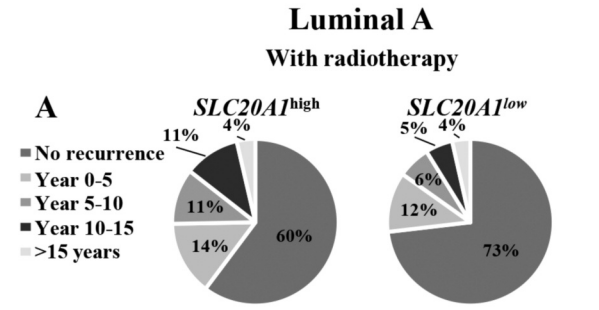
Patients with luminal A breast cancer in the SLC20A1^{high} group with radiotherapy have a high risk of late recurrence. Late recurrence is one of the significant clinical problems of ER+ breast cancer (27-33). Some patients with luminal A and luminal B breast cancer relapse after the end of long-term therapy, although patients with luminal A and luminal B breast cancer have intrinsically better prognoses than those with other subtypes. Although late recurrence of breast cancer has been examined focusing on endocrine therapy (27, 29, 32, 33), late recurrence for radiotherapy is unclear (34). Therefore, the present study analyzed the recurrence incidence rate and rate ratio every 5 years from the time of diagnosis for patients in the *SLC20A1*^{high} and *SLC20A1*^{low} groups with luminal A and luminal B breast cancer with radiotherapy. Pie charts of the recurrence period and the numbers of patients in the *SLC20A1*^{high} and *SLC20A1*^{low} groups with luminal A and luminal B breast cancer with radiotherapy are shown (Figure 4A and B). Among patients with luminal A breast cancer with radiotherapy, patients in the *SLC20A1*^{high} group did not markedly differ from patients in the *SLC20A1*^{low} group at 0-5 years in terms of the recurrence incidence rate. At 5-10 years, patients in the *SLC20A1*^{high} group had a slightly higher recurrence incidence rate than those at 0-5 years. At 10-15 years, patients in the *SLC20A1*^{high} group tended to show a higher recurrence incidence rate than patients in the *SLC20A1*^{low} group. At >15 years, patients in the *SLC20A1*^{low} group showed a slightly higher recurrence incidence rate than patients in the *SLC20A1*^{low} group for other time periods; however, patients in the *SLC20A1*^{high} group had a higher recurrence incidence rate than patients in the *SLC20A1*^{low} group at >15 years (Year 0-5: Incidence rate ratio=1.30, 95%CI=0.67-2.51; Year 5-10: Incidence rate ratio=1.90, 95%CI=0.85-4.24; Year 10-15: Incidence rate ratio=2.43, 95%CI=1.06-5.56; >15 years: Incidence rate ratio=1.60, 95%CI=0.44-5.81) (Figure 4C). Importantly, although the rate differences were not statistically significant, the recurrence incidence rates for *SLC20A1*^{high} patients were higher than those for *SLC20A1*^{low} patients at all periods in luminal A breast cancer. Patients with luminal B breast cancer in the *SLC20A1*^{high} group tended to show a higher recurrence incidence rate than patients in the *SLC20A1*^{low} group for all periods (Year 0-5: Incidence rate ratio=1.16, 95%CI=0.76-1.77; Year 5-10: Incidence rate ratio=1.72, 95%CI=0.94-3.15; Year 10-15: Incidence rate ratio=2.70, 95%CI=0.88-8.26; >15 years: Incidence rate ratio=2.18, 95%CI=0.14-34.89) (Figure 4D). On the other hand, patients with luminal B breast cancer in both the *SLC20A1*^{high} and *SLC20A1*^{low} groups showed the highest recurrence incidence rate at 0-5 years. The curve of the recurrence incidence rate decreased as the period progressed. Therefore, these results indicate that high expression levels of *SLC20A1* may be involved in the late recurrence of luminal A tumors after radiotherapy.

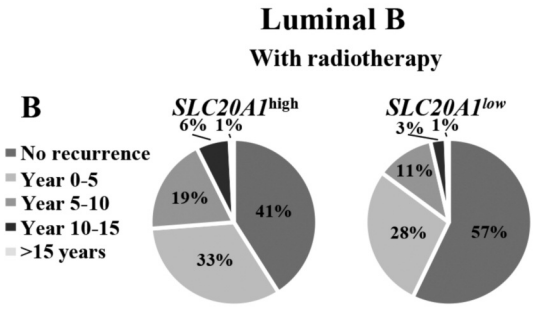
Discussion

The present study revealed that patients in the *SLC20A1*^{high} group with luminal A breast cancer with radiotherapy showed poor clinical outcomes and had a short interval until recurrence (Figure 1 and Table I). Patients in the *SLC20A1*^{high} group with luminal B breast cancer with radiotherapy also showed poor clinical outcomes and had a short interval until recurrence, except for in multivariate Cox regression analysis of DSS (Figure 1 and Table I). Our previous study already reported that, in claudin-low and basal-like breast cancer, patients in the *SLC20A1*^{high} group with radiotherapy show poor clinical outcomes (20). Therefore, radiotherapy is insufficient for patients in the *SLC20A1*^{high} group for not only the claudin-low and basal-like types but also for the luminal A and luminal B types. Thus, the SLC20A1-dependent response to X-ray may be required for the acquirement of resistance to radiotherapy in luminal A, luminal B, claudin-low, and basal-like breast cancer.

Apart from the METABRIC dataset, the dataset from The Cancer Genome Atlas (TCGA) (35) is also a valuable cancer genomics dataset containing data on radiotherapy and was also downloaded to examine the prognoses of patients in the *SLC20A1*^{high} group with radiotherapy in terms of DSS, disease-free status (DFS), and progress-free status in patients with luminal A and luminal B breast cancer compared with patients in the *SLC20A1*^{low} group. TCGA dataset did not show results same to those of METABRIC dataset. The TCGA dataset is a small population, often contains censored data, and only covers ~10 years of observation period, in comparison with the METABRIC dataset containing a bigger population and covering ~20 years of observation period. Therefore, the differences between the results may reflect these differences between the TCGA and METABRIC datasets.

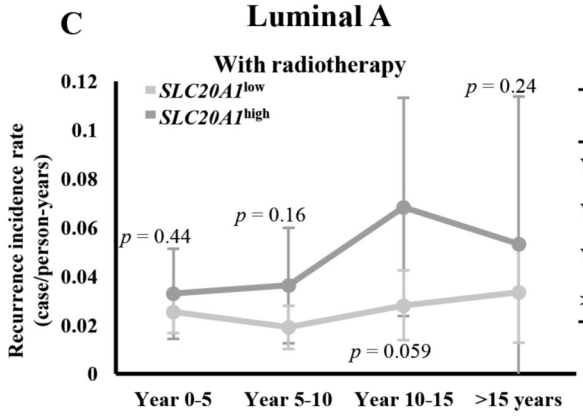
At tumor stage I, patients in the *SLC20A1*^{high} group with luminal A breast cancer with radiotherapy showed poor clinical outcomes (Figure 2B and Figure 3B). However, at tumor stage II, there were no significant differences between patients in the *SLC20A1*^{high} and *SLC20A1*^{low} groups with luminal A breast cancer with radiotherapy (Figure 2F and Figure 3F). These results indicate that radiotherapy for



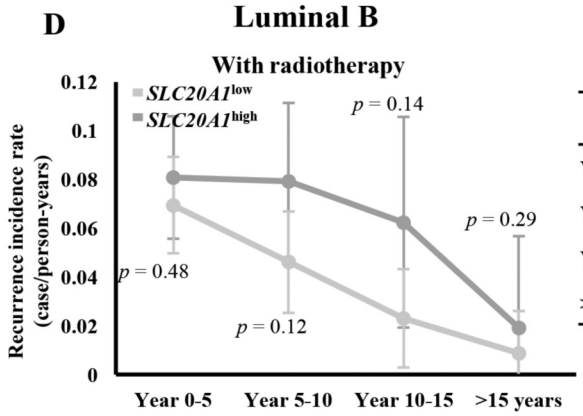


Luminal A with radiotherapy	*SLC20A1*^{high} (n =)	*SLC20A1*^{low} (n =)
No recurrence	50	207
Year 0-5	12	33
Year 5-10	9	18
Year 10-15	9	15
>15years	3	10

Luminal B with radiotherapy	*SLC20A1*^{high} (n =)	*SLC20A1*^{low} (n =)
No recurrence	50	96
Year 0-5	40	47
Year 5-10	23	19
Year 10-15	8	5
>15years	1	1



	Incidence rate ratio	95%Confidence interval			*SLC20A1*^{high} (n=)	*SLC20A1*^{low} (n=)
Year 0-5	1.30	0.67	-	2.51	21	57
Year 5-10	1.90	0.85	-	4.24	25	77
Year 10-15	2.43	1.06	-	5.56	18	69
>15 years	1.60	0.44	-	5.81	19	80



	Incidence rate ratio	95%Confidence interval			*SLC20A1*^{high} (n=)	*SLC20A1*^{low} (n=)
Year 0-5	1.16	0.76	-	1.77	44	63
Year 5-10	1.72	0.94	-	3.15	39	43
Year 10-15	2.70	0.88	-	8.26	25	35
>15 years	2.18	0.14	-	34.89	14	27

Figure 4. *High solute carrier family 20 member 1 (SLC20A1) expression (SLC20A1^{high}) is associated with higher recurrence incidence rates compared with those of patients with low SLC20A1 expression (SLC20A1^{low}) at years 10-15 in luminal A breast cancer. (A-B) Pie charts of the recurrence period and numbers of patients in the SLC20A1^{high} and SLC20A1^{low} groups among patients with luminal A or luminal B breast cancer who were subjected to radiotherapy. (A) Patients with SLC20A1^{high} (left) and SLC20A1^{low} (right) luminal A breast cancer. (B) Patients with SLC20A1^{high} (left) and SLC20A1^{low} (right) luminal B breast cancer. (C-D) Line graphs showing the recurrence incidence rates of patients in the SLC20A1^{high} and SLC20A1^{low} luminal A or luminal B groups every 5 years with or without radiotherapy. p-values were calculated using the statistical analysis based on normal distribution and corrected using the Holm method. The incidence rate ratio was calculated as the ratio of the recurrence incidence rate of the SLC20A1^{high} group to that of the SLC20A1^{low} group. The 95% confidence interval and number of patients are shown on the right. (C) Patients with luminal A breast cancer. (D) Patients with luminal B breast cancer.*

patients in the *SLC20A1*^{high} group with luminal A breast cancer at tumor stage I is insufficient but that for patients in the *SLC20A1*^{high} group with luminal A breast cancer at tumor stage II is sufficient. Since X-ray damage induces Rad51-dependent DNA repair, which is necessary for homologous recombination restoration in proliferating cells rather than resting cells (36), the different effects of radiotherapy may reflect that stage II tumors have a higher proliferative profile than tumors of tumor stage I. Therefore, it is important to consider not only the difference in *SLC20A1* gene expression but also that in tumor stage when selecting radiotherapy.

It has been reported that breast cancer cell lines have different sensitivities to radiation and their sensitivities are not associated with their respective intrinsic subtype (15). The results of the present study suggest that patients in the *SLC20A1*^{high} group with both luminal A and luminal B breast cancer are radiotherapy-resistant, and that the current classification by PAM50 or immunohistochemistry is not enough for radiotherapy. Therefore, SLC20A1 gene expression may be a novel biomarker for classification to select radiotherapy against ER+ breast cancer.

SLC20A1 is an inorganic phosphate (Pi) symporter and increases Pi uptake contributing to DNA synthesis and the regulation of the cell cycle (18, 37, 38). Furthermore, radiotherapy induces DNA damage of cancer cells and cell death (15, 36, 39, 40). Therefore, SLC20A1-dependent Pi uptake may be involved in DNA repair against X-ray damage and acquirement of radiotherapy resistance. Thus, it would also be necessary to analyze the mechanism of acquiring radiotherapy resistance in patients in the *SLC20A1*^{high} group with luminal A and luminal B breast cancer.

Late recurrence is one of the significant clinical problems in ER+ breast cancer. In a previous study, patients with breast cancer with radiotherapy had a high odds ratio of >5-year recurrence in comparison with patients having mastectomy without radiotherapy, although patients with radiotherapy had a high odds ratio of <5-year recurrence (34). Late recurrence is associated with dormancy and cancer stem cells (41-44). Furthermore, radiation induces cell cycle arrest in luminal A type MCF-7 cells (39) and breast cancer stem cells have radiation resistance (8, 40). SLC20A1 deficiency suppresses cell viability in MCF-7 cells (20). In addition, SLC20A1 deficiency suppresses tumor-sphere formation by ALDH1^{high} cells and the viability of claudin-low type MDA-MB 231 cells and basal-like type MDA-MB 468 cells (20). Patients in the *SLC20A1*^{high} group with luminal A breast cancer with radiotherapy tended to have a high recurrence incidence rate at years 10-15 (Figure 4C), and patients in the *SLC20A1*^{high} group with luminal B breast cancer with radiotherapy tended to have a high recurrence incidence rate at all periods and the recurrence incidence rate decreased as the period progressed (Figure 4D). Therefore, high *SLC20A1* expression may induce late recurrence in patients with luminal A breast cancer with radiotherapy; however, in patients with luminal B breast cancer with radiotherapy, high *SLC20A1* expression is involved in recurrence regardless of the period. A significant proportion of breast cancer cells in the luminal B subtype is HER2-positive. HER2-positive cancer is highly proliferative, enriched in cancer stem cells, and resistant to irradiation (45). These results may reflect these differentiations between luminal A and luminal B types. However, our previous study showed that Her2-enriched breast cancer with *SLC20A1*^{high} had a better prognosis compared with *SLC20A1*^{low} breast cancer, and both claudin-low and basal-like breast cancers with *SLC20A1*^{high} correlated with poor prognosis (20). Taken together with the above mentions, SLC20A1 may independently contribute to ER and HER2 signaling in cancerous progression. In addition, because endocrine therapy is also the main treatment for ER+ patients, it would be necessary to analyze the recurrence incidence rate of patients in the *SLC20A1*^{high} group with endocrine therapy.

Conflicts of Interest

The Authors state that they have no conflicts of interest to declare in regard to this study.

Authors' Contributions

C.O., S.T., I.M. and Ka.S. performed the analyses; C.O., S.T. and K.A. conceived the study; C.O. drafted the manuscript; C.O., S.T., I.M., A.O., H.M., Yu.N., T.S., Ke.S., K.T., Y.X., Yo.N., Y.M., S.M., Ka.S., S.O. and K.A. contributed to discussion and review of the final manuscript; all the Authors approved the final manuscript.

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