Breast Cancer: Targets and Therapy

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ORIGINAL RESEARCH

Metabolomics in Radiotherapy-Induced Early Adverse Skin Reactions of Breast Cancer Patients

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Background: Early adverse skin reactions (EASRs) are common side effects of radiotherapy (RT) that impact the quality of life of breast cancer patients. This study used global metabolomics profiles of breast cancer populations to identify metabolic pathways and biomarkers significantly associated with RT-induced EASRs to identify potential targets for precision interventions.

Methods: We used a frequency-matched study design to identify pre-RT urine samples from 60 female breast cancer patients (30 with high and 30 with low EASRs) for metabolomic analysis by Metabolon Inc. using UPLC-MS/MS and GC-MS. Using MetaboAnalyst, we performed metabolomic data analysis and visualization on 84 candidate metabolites from 478 total compounds. We used the Oncology Nursing Society (ONS) Skin Toxicity Criteria (0–6) for EASRs assessment.

Results: Seven metabolic pathways were significantly associated with RT-induced EASRs, including alanine, aspartate, and glutamate metabolism ($p = 0.0028$), caffeine metabolism ($p = 0.0360$), pentose and glucuronate interconversions ($p = 0.0028$), glycine, serine, and threonine metabolism ($p = 0.0360$), beta-alanine metabolism ($p = 0.0210$), pantothenate and CoA biosynthesis ($p = 0.0028$), and glutathione metabolism ($p = 0.0490$). The alanine, aspartate, and glutamate metabolic pathway had the lowest false discovery rate (FDR)-adjusted p-value and the highest impact value of 0.60. Thirteen metabolite biomarkers were significantly associated with RT-induced EASRs.

Conclusion: Our data show that the alanine, aspartate, and glutamate metabolism pathways had the highest impact value on RT-induced EASRs. Future larger studies are warranted to validate our findings and facilitate targeted interventions for preventing or mitigating RT-induced EASRs, offering a promising direction for further research and clinical applications.

Keywords: breast cancer, metabolomics, radiotherapy, early adverse skin reactions

Background

Breast cancer is one of the most frequently diagnosed cancers and the second leading cause of cancer death among women. With over 4 million survivors, 313,510 new cases, and 42,780 deaths expected in 2024, research on breast cancer treatment, survival, and quality of life (QOL) remains critical.[1] For early-stage tumors, the standard treatment consists of breast-conserving surgery, also known as lumpectomy or partial mastectomy, resulting in improved cosmetic effects and comparable survival rates to conventional mastectomy procedures.[2] Most patients undergoing breast-conserving surgery receive postoperative radiotherapy (RT) to reduce the risk of loco-regional recurrence and improve survival.[3] Although adjuvant RT is well tolerated, many patients experience acute and late side effects that negatively impact the QOL.[4] Thus, it is important to address RT-related early adverse skin reactions (EASRs) that significantly impact the QOL of breast cancer survivors.[5]

One of the most frequent and burdensome side effects of postoperative breast RT is EASRs[6] resulting in itching, soreness, peeling, and burning sensations in affected patients. EASRs affect the majority of patients receiving RT, with many reporting skin changes during treatment, ranging from mild erythema to moist desquamation and ulceration in

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more severe cases.[7] With a high prevalence and variance in clinical presentations and severity of EASRs,[8] it is important to evaluate underlying factors that place patients at a heightened risk for severe reactions. Several clinical variables, such as body mass index (BMI), age, smoking, breast volume, race/ethnicity, postmenopausal status, and inhomogeneity of dose[9–12] have been associated with EASRs, and a growing body of evidence suggests radio-sensitivity results from a combination of complex genetic risk factors and biological pathways.[13] Newer treatment protocols including 5-fraction partial breast irradiation and ultra-hypofractionated whole-breast irradiation[14,15] may be associated with milder EASR; however, not all patients are eligible for these approaches, and EASRs are still a common toxicity. Recent studies have begun to explore the biological mechanisms and pathways involved in the development of RT-induced skin toxicities in breast cancer populations, including genetic mutations and inflammatory cytokine genes.[16,17] So far, our past studies reported that elevated levels of the inflammatory biomarker C-reactive protein and polymorphisms in DNA damage repair genes may be associated with EASRs in breast cancer patients.[18,19]

Metabolomics, a growing field of studying metabolites, could provide insight into biological pathways associated with RT-induced EASRs. Metabolomics reflects overall change within biological systems; metabolites, the end products of genomic, transcriptomic, and proteomic processes, are closely connected to the functions and characteristics of cells and tissues.[20] With the expansive advancements in metabolomics-driven technologies, researchers have begun to uncover metabolic pathways and biomarkers of interest in breast cancer risk, treatment responses, and survival revealing interactions of tumor cells and various biological molecules, including lipids, amino acids, and organic acids.[21–24] There is, however, limited literature exploring the potential contribution of metabolic processes to RT-induced EASRs. Therefore, in the present study, we evaluated global metabolomics across multiple biological pathways in the development of RT-induced EASRs with the intent to identify potential targets for precision interventions.

Methods

Study Population

Patients were recruited from the University of Miami Sylvester Comprehensive Cancer Center (SCCC) and Jackson Memorial Hospital (JMH), Florida, in the United States from 2008 to 2014. Female patients (age ≥18) newly diagnosed with breast carcinoma, Stage 0–III were recruited before the initiation of RT. The study was approved by the University of Miami's Institutional Review Board and written informed consent was obtained from each participant after providing a detailed description of the protocol in English or Spanish. For the current metabolomics study, 60 patients were randomly selected from the parent study, which was previously reported elsewhere,[25] based on EASR grade (30 with a low grade and 30 with a high grade) and were frequency matched by race/ethnicity and BMI. Urine samples were collected before and after RT.

RT-Induced EASRs Assessment

Participants were administered adjuvant RT in the supine position to the whole breast using standard opposed tangential fields or to the whole breast and regional lymph nodes at the supervising physician's discretion. Participants received either conventional RT (2.0 Gy/day over 5–6 weeks, mostly 50 Gy in 25 fractions) or hypo-fractionated RT (>2.0 Gy/day over 3 weeks, most commonly 42.4 Gy in 16 fractions).[25] In some cases, a boost dose of 10 to 16 Gy was delivered to the lumpectomy cavity. After the conclusion of RT, EASRs were evaluated by the attending physician using ONS Skin Toxicity Criteria. The ONS scale divides EASRs into seven categories (grade 0–6): 0 - No changes noted; 1 - faint or dull erythema and/or follicular reaction and/or itching; 2 - bright erythema and/or tender to touch; 3 - dry desquamation with or without erythema; 4 - small or moderate amount of wet desquamation; 5 - confluent moist desquamation; 6 - ulceration, hemorrhage, and/or necrosis. ONS grades 0 and 1 indicate a low-grade EASR while ONS grade 4+ indicates a high-grade EASR.

Metabolomic Profiling

Urine samples were collected before and after radiotherapy (RT), immediately stored at −80°C and then processed at Metabolon, Inc. Urine metabolites were processed using liquid chromatography/mass spectrometry and analyzed by

Metabolon, Inc. (Morrisville, USA). The platform prepared samples using the MicroLab STAR® system.[26] Recovery standards were added for quality control (QC) before the extractions. During the extractions, samples underwent a series of organic and aqueous extractions to eliminate the protein fraction while maximally preserving small molecules. The resulting extract was aliquoted in two divisions: one for UPLC-MS/MS analysis and one for GC/MS analysis. Samples were then vortexed on a TurboVap® (Zymark) to evaporate the organic solvent. Finally, each sample was freeze-dried by vacuum and prepared for either LC/MS-LC/MS or GC/MS. Internal and derivatization standards were employed for QC purposes. The raw data outputs were archived, extracted, and accessioned into the Metabolon Library Information Management System (LIMS) for metabolomic profiling. The informatics system included LIMS, data extraction and peak-identification software, data processing tools for QC and compound identification, and interpretation and visualization tools for enhanced reporting and data analysis. Normalization steps were achieved to correct inter-day tuning differences between instruments employed in multiple-day studies. Compound concentrations were normalized by equating the run-day medians to one (1.00) and plotting each data point proportionately.

Statistical Analysis

Student's *t*-tests were performed on pre-RT metabolite levels to compute differences in metabolite levels of the 478 total compounds between skin toxicity groups of low vs high EASRs. A *p*-value threshold of 0.2 was used to identify the candidate metabolites for pathway analysis. Enrichment and topology analyses were conducted on the 84 candidate metabolites to identify pathways associated with RT-induced EASRs. Pathway enrichment analysis employed hypergeometric tests to identify compounds overrepresented in their respective pathways, while pathway topology analysis evaluated the relative betweenness centrality, the proportion of shortest paths passing through a node, to yield pathway impact values (IVs).[27] Point-biserial correlation coefficients were calculated as measures of the linear relationship between pre-RT metabolite levels and skin toxicity groups. False discovery rate (FDR)-adjusted *p*-values are controlled for multiple testing. Pathway enrichment and topology analyses were performed using MetaboAnalyst 3.0 (www.metaboanalyst.ca). Other analyses were performed using SAS v. 9.4 (SAS Inc., Cary, NC).

Results

Table 1 summarizes the patient tumor and clinical characteristics of the study population. The nested population consisted of 24 Black or African American (40%), 18 non-Hispanic White (30%), 14 Hispanic White (23%), and 4 others (7%), as well as 4 normal weight (7%), 4 overweight (7%), and 52 (87%) obese patients. Twenty-seven of the sixty patients (45%) were older than 60 years of age. Fifty-six patients (93%) received a conventional RT dosage based on prevailing practices at that time.

Table 2 reports for 25 metabolites, the correlations between pre-RT metabolite levels and skin toxicity groups and reports the pre-RT metabolite means (and standard deviations) by skin toxicity groups. These 25 metabolites

Table 1 Selected Characteristics of the Study Population by EASR Status

Variables	Categories	Total Patients	%	Low EASR (ONS Grade 0–1)	%	High EASR (ONS Grade 4+)	%
Total		60	100%	30	100%	30	100%
Race/ Ethnicity	AA	24	40%	12	40%	12	40%
	HW	14	23%	7	23%	7	23%
	NHW	18	30%	9	30%	9	30%
	Other	4	7%	2	7%	2	7%
BMI class	Normal	4	7%	2	7%	2	7%
	Overweight	4	7%	2	7%	2	7%
	Obese	52	87%	26	87%	26	87%
Age	< 60	33	55%	12	40%	21	70%

(*Continued*)

Table 1 (Continued).

Variables	Categories	Total Patients	%	Low EASR (ONS Grade 0–1)	%	High EASR (ONS Grade 4+)	%
	≥ 60	27	45%	18	60%	9	30%
ER	Positive	47	78%	25	83%	22	73%
	Negative	13	22%	5	17%	8	27%
PR	Positive	43	72%	21	70%	22	73%
	Negative	17	28%	9	30%	8	27%
Triple negative	No	52	87%	27	90%	25	83%
	Yes	8	13%	3	10%	5	17%
Tumor stage	0	9	15%	4	13%	5	17%
	1	30	50%	16	53%	14	47%
	2	19	32%	10	33%	9	30%
	3A	2	3%	0	0%	2	7%
Prior chemo	No	49	82%	26	87%	23	77%
	Yes	11	18%	4	13%	7	23%
RT type	Conventional	56	93%	27	90%	29	97%
	Hypo-fractionated	4	7%	3	10%	1	3%

Note: no group difference due to frequency-matching.
Abbreviations: AA, Black or African American; HW, Hispanic white; NHW, non-Hispanic white; EASR, early adverse skin reaction; BMI, body mass index; ER, estrogen receptor; PR, progesterone receptor; RT, radiotherapy.

Table 2 Pre-RT Metabolites That Differ by RT-Induced EASRs Status

Metabolites	Pathway	Correlation	*P*	Low EASRs		High EASRs		*P*[a]
				Mean	SD	Mean	SD	
Ethanolamine	Phospholipid Metabolism	0.384	0.002	0.80	0.38	1.14	0.44	0.002
Thymine	Pyrimidine Metabolism, Thymine containing	0.320	0.013	0.86	0.33	1.09	0.37	0.013
3-ureidopropionate	Pyrimidine Metabolism, Uracil containing	0.312	0.015	1.04	0.39	1.40	0.69	0.016
Glucuronate	Amino-sugar Metabolism	0.297	0.021	0.96	0.52	1.39	0.86	0.022
Cinnamoylglycine	Food Component/Plant	0.298	0.021	0.94	0.75	2.36	3.19	0.023
Fucose	Pentose Metabolism	0.286	0.027	0.96	0.51	1.31	0.66	0.027
Orotate	Pyrimidine Metabolism, Orotate containing	0.281	0.029	0.92	0.42	1.29	0.82	0.031
Uracil	Pyrimidine Metabolism, Uracil containing	0.280	0.030	0.87	0.43	1.19	0.63	0.031
Ribulose	Pentose Metabolism	0.275	0.033	0.99	0.43	1.27	0.54	0.033
Glutamate	Glutamate Metabolism	0.274	0.034	0.86	0.50	1.20	0.69	0.034
Alanine	Alanine and Aspartate Metabolism	0.271	0.036	0.88	0.35	1.16	0.62	0.037
Gamma-aminobutyrate	Glutamate Metabolism	0.270	0.037	0.81	0.52	1.27	1.05	0.038
Glucose	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	0.274	0.034	0.95	0.49	6.55	14.2	0.039
Cortolone	Steroid	0.249	0.055	0.86	0.54	1.27	1.03	0.057
2-hydroxyphenylacetate	Phenylalanine and Tyrosine Metabolism	0.249	0.055	0.99	0.32	1.09	0.44	0.060
Inosine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	0.241	0.064	0.90	0.42	1.12	0.46	0.064
Gamma-CEHC	Tocopherol Metabolism	0.241	0.063	0.56	0.49	0.68	0.69	0.064
5,6-dihydrouracil	Pyrimidine Metabolism, Uracil containing	0.239	0.066	0.86	0.37	1.07	0.47	0.065
4-methyl-2-oxopentanoate	Leucine, Isoleucine and Valine Metabolism	0.239	0.066	0.72	0.32	1.03	0.84	0.069
3-methoxy-4-hydroxyphenylglycol	Phenylalanine and Tyrosine Metabolism	0.233	0.073	0.92	0.51	1.18	0.59	0.073
Glucose-6-phosphate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	0.231	0.075	0.66	0.49	1.59	2.77	0.080
N-acetylmannosamine	Amino sugar Metabolism	0.224	0.085	0.89	0.84	1.16	1.41	0.086
3-hydroxycotinine glucuronide	Tobacco Metabolite	0.211	0.106	0.40	0.12	0.60	0.62	0.111
Aspartate	Alanine and Aspartate Metabolism	0.189	0.148	1.01	0.61	1.39	1.29	0.150
Putrescine	Polyamine Metabolism	0.184	0.160	1.13	1.37	1.85	2.43	0.161

Notes: Correlation: Point-Biserial correlation between pre-RT metabolite level and skin toxicity low and high EASR groups. [a]Student's *t*-test comparing pre-RT metabolite mean between low and high EASR groups.
Abbreviations: *EASRs*, early adverse skin reactions; *SD*, standard deviation; P, p-value.

Table 3 Metabolic Pathways That Differ by RT-Induced EASR Status at FDR<0.20

Significant Pathways	Total	Expected	Hits	Raw p^a	-log(p)	Holm- adjusted p	FDR p	Impact[b]
Alanine, aspartate and glutamate metabolism	24	0.7	6	4.34×10^{-5}	1.00×10^{1}	3.47×10^{-3}	2.83×10^{-3}	0.60
Pantothenate and CoA biosynthesis	27	0.79	6	8.91×10^{-5}	9.33 x10°	7.04×10^{-3}	2.83×10^{-3}	0.06
Pentose and glucuronate interconversions	53	1.54	8	1.06×10^{-4}	9.15x10°	8.29×10^{-3}	2.83×10^{-3}	0.15
Beta-Alanine metabolism	28	0.81	5	1.05×10^{-3}	6.86x10°	8.10×10^{-2}	2.10×10^{-2}	0.07
Glycine, serine and threonine metabolism	48	1.4	6	2.28×10^{-3}	6.08x10°	1.73×10^{-1}	3.60×10^{-2}	0.11
Caffeine metabolism	21	0.61	4	2.70×10^{-3}	5.91x10°	2.03×10^{-1}	3.60×10^{-2}	0.29
Glutathione metabolism	38	1.11	5	4.29×10^{-3}	5.45x10°	3.17×10^{-1}	4.90×10^{-2}	0.04
Arginine and proline metabolism	77	2.24	7	6.17×10^{-3}	5.19x10°	4.51×10^{-1}	6.17×10^{-2}	0.11
Ascorbate and aldarate metabolism	45	1.31	5	8.91×10^{-3}	4.72x10°	6.42×10^{-1}	7.92×10^{-2}	0.17
Taurine and hypotaurine metabolism	20	0.58	3	1.88×10^{-2}	3.97x10°	1.00x10°	1.37×10^{-1}	0.05
Citrate cycle (TCA cycle)	20	0.58	3	1.88×10^{-2}	3.97x10°	1.00x10°	1.37×10^{-1}	0.17
Cysteine and methionine metabolism	56	1.63	5	2.18×10^{-2}	3.83x10°	1.00x10°	1.45×10^{-1}	0.17
Butanoate metabolism	40	1.16	4	2.72×10^{-2}	3.60x10°	1.00x10°	1.63×10^{-1}	0.11
Pyrimidine metabolism	60	1.74	5	2.85×10^{-2}	3.56x10°	1.00x10°	1.63×10^{-1}	0.17
Histidine metabolism	44	1.28	4	3.71×10^{-2}	3.29x10°	1.00x10°	1.86×10^{-1}	0.05
Phenylalanine metabolism	45	1.31	4	3.99×10^{-2}	3.22x10°	1.00x10°	1.86×10^{-1}	0.01
Amino sugar and nucleotide sugar metabolism	88	2.56	6	4.04×10^{-2}	3.21x10°	1.00x10°	1.86×10^{-1}	0.11
Valine, leucine and isoleucine biosynthesis	27	0.79	3	4.18×10^{-2}	3.17x10°	1.00x10°	1.86×10^{-1}	0.06

Notes: Total, number of compounds in pathway; Hits, matched number of compounds from uploaded data. [a]Pathway enrichment analysis. [b]Pathway topology analysis.
Abbreviation: FDR, false discovery rate.

were included in the pathway analysis using a *p*-value threshold of 0.20. The top 13 metabolites listed differ significantly at $p < 0.05$ between low and high EASR groups; these were ethanolamine, thymine, 3-ureidopropionate, glucuronate, cinnamoylglycine, fucose, orotate, uracil, ribulose, glutamate, alanine, gamma-aminobutyrate, and glucose. Various carbohydrates including glucose ($r = 0.274$; $p = 0.034$), ribulose ($r = 0.275$; $p = 0.033$), fucose ($r = 0.286$; $p = 0.027$), and glucuronate ($r = 0.297$; $r = 0.021$) showed positive correlations between pre-RT metabolite level and skin toxicity, and all showed statistically significant higher mean pre-RT metabolite levels in high EASR group compared to low EASR group. Similar positive correlations between pre-RT metabolite levels and skin toxicity groups were observed for metabolites involved in pyrimidine metabolisms such as uracil ($r = 0.280$; $p = 0.030$), 3-ureidopropionate ($r = 0.312$; $p = 0.015$), orotate ($r = 0.281$; $p = 0.029$), and thymine ($r = 0.320$; $p = 0.013$), as well as compounds such as alanine ($r = 0.271$; $p = 0.036$), ethanolamine ($r = 0.384$; $p = 0.002$), cinnamoyl glycine ($r = 0.298$; $p = 0.021$) and gamma-aminobutyrate ($r = 0.270$; $p = 0.037$); and for all these metabolites, mean pre-RT metabolite level was higher in the high EASR group. Of special interest was the molecule glutamate, showing pre-RT levels positively correlated with skin toxicity ($r = 0.274$, $p = 0.034$; mean 0.86 vs 1.20, in low and high EASR groups, respectively, $p = 0.034$).

Metabolic Pathways Associated with RT-Induced EASRs

Pathway enrichment and topology analyses identified pathways associated with RT-induced EASRs. Table 3 summarizes the results, presenting the expected and the actual number of hits, FDR-adjusted *p*-values, and impact values (IV) for the pathways. The following seven metabolic pathways were significantly associated with RT-induced EASRs: alanine, aspartate, and glutamate metabolism ($p = 0.0028$; IV = 0.60); caffeine metabolism ($p = 0.0360$; IV = 0.29); pentose and glucuronate interconversions ($p = 0.0028$; IV = 0.15); glycine, serine, and threonine metabolism ($p = 0.0360$; IV = 0.11); beta-alanine metabolism ($p = 0.0210$; IV = 0.07); pantothenate and CoA biosynthesis ($p = 0.0028$; IV = 0.06); and glutathione metabolism ($p = 0.0490$; IV = 0.04). Alanine, aspartate, and glutamate metabolism had the most significant FDR *p*-value and the highest IV in predicting RT-induced EASRs among the pathways observed.

Discussion

As postoperative breast RT remains a standard postoperative treatment for many breast cancer survivors, it is important to thoroughly evaluate the adverse effects associated with this practice. Although previous studies suggest that the severity and symptomatology of EASRs differ by patient disease and treatment characteristics,[19,28] there is generally limited knowledge on the role of metabolic pathways and biomarkers in the development of RT-induced EASRs. Our study addresses the dearth of literature on the biological mechanisms of EASRs by capitalizing on advancements in metabolomic profiling to evaluate biomarkers associated with these reactions. As EASRs significantly affect the QOL of breast cancer survivors, our findings target an important gap in breast RT research and offer insight into potential precision medicine targets for RT-induced adverse reactions.

In our pilot study of 60 breast cancer patients, seven metabolic pathways were significantly associated with RT-induced EASRs after adjusting for race/ethnicity and BMIs. Among those pathways, the alanine, aspartate, and glutamate metabolism pathways had the highest IV at 0.60 with an FDR-adjusted *p*-value of 0.0028, representing the highest cumulative percentage of matched metabolite nodes exhibiting pathway importance by relative betweenness centrality. This finding enhances preliminary evidence of the importance of glutamate metabolism in RT-induced EASRs supporting the exploration of novel clinical interventions targeting glutaminase.[29] Additional pathways including pantothenate and CoA biosynthesis, pentose and glucuronate interconversions, beta-alanine metabolism, glycine, serine, and threonine metabolism, caffeine metabolism, and glutathione metabolism were associated with RT-induced EASRs; however, the IVs of these pathways were lower than that of glutamate ranging from 0.04 to 0.29. Thus, alanine, aspartate, and glutamate metabolism emerged as a pathway of interest in the development of RT-induced EASRs.

Our interest in glutamate metabolism was further enhanced by the identification of glutamate as a significant metabolite in the correlation with RT-induced EASRs ($r = 0.274$; $p = 0.034$). Glutamate, the most abundant amino acid in the human body, plays a crucial role in multiple physiological processes including cognition, learning, and behavior, and high levels of production can lead to neuronal dysfunction.[30] More importantly, glutamate plays a critical role in tumor bioenergetics serving as a growth factor and signal mediator in both neuronal and non-neuronal neoplasms.[31] Past research has shown that hyperglycemic conditions promote more aggressive phenotype in various cancers, including breast,[32] and there is growing evidence to suggest that glutaminase inhibition may enhance radiosensitivity.[33,34] Our glutamate-related findings are supported by previous studies that revealed the influence of RT on serum/plasma concentrations of various energy-related metabolites in patients with breast cancer.[35,36] Aside from RT-induced changes, the potential target role of glutamate in breast cancer therapy has been furthered by studies exploring the importance of estrogen-mediated glutamate-signaling to the aggressiveness of triple-negative breast cancer, as well as the role of glutamine-signaling in cancer-induced pain.[37,38] With recent expansions in clinical applications of glutaminase inhibitors and a clinically available glutaminase inhibitor, telaglenastat (CB-839),[39] this may be a promising treatment option to prevent RT-induced EASRs while also capitalizing on their anticancer mechanisms to improve treatment outcomes.[29,40]

In addition to glutamate, pre-RT levels of 12 metabolites including ethanolamine, thymine, 3-ureidopropionate, glucuronate, cinnamoyl glycine, fucose, orotate, uracil, ribulose, alanine, gamma-aminobutyrate, and glucose were significantly higher in the high EASR group. Changes in metabolite concentrations of carbohydrates, including glucose, pentose, ribulose, fucose, and glucuronate, were positively correlated with RT-induced EASRs. As high levels of fucose and glucuronate are linked to the degradation and recycling of extracellular glycoproteins and glycosaminoglycans, they may play critical roles in the impairment of skin repair.[41,42] Whereas elevated levels of nucleic acid metabolites, such as uracil and 3-ureidopropionate and orotate may reflect the breakdown of pyrimidine nucleotides required for tissue recovery.[43] Our findings are supported by previous studies reporting significant changes from pre- to post-RT in serum/plasma metabolome profiles, including levels of phospholipids as well as metabolites involved in glycolysis, citric acid cycle, and amino acid metabolism.[35,36,44,45] Furthermore, our findings contribute to the literature suggesting that microbial metabolites modulate immune pathways and tissue injury and repair mechanisms[46] and that the microbiome may impact the efficacy and toxicity of anti-cancer therapeutics, including radiotherapy.[47] The growing body of evidence illustrates the importance of metabolites in predicting RT-induced changes, encouraging future longitudinal studies of metabolomics-based modeling in RT-

induced EASRs. As technological advancements in artificial intelligence continue, there may be increasing opportunities to leverage pre-RT metabolome profiles to develop predictive algorithms for EASRs.[48,49]

This study had several strengths and weaknesses. First, we used a prospective study design to capture the development of RT-induced EASRs, which is impossible with a cross-sectional or retrospective study design. Second, the study findings capitalized on recent advancements in metabolomics-based technologies by employing liquid chromatography-mass spectrometry (LC-MS), and gas chromatography (GC-MS) to profile biological compounds of interest in the development of RT-induced EASRs. The collateral use of LC-MS and GC-MS has led to improved compound profiling capacity along with advancements in the sensitivity and selectivity platform of MS which enhanced metabolic profiling in recent years.[50] Third, this is one of the first studies to use metabolomics-based analyses to evaluate radiosensitivity in breast cancer populations, and our findings demonstrate potential applications of metabolomics to study RT-induced EASRs and related QOL in cancer patients receiving adjuvant RT. The major limitation of this study is the relatively small sample size (n=60). To overcome this limitation to some extent, we employed a frequency-matched study design to select samples (30 low and 30 high EASRs). It is also important to recognize that many patients are now able to receive moderately or ultra-hypofractionated protocols, which may have less skin toxicity. Future larger studies are warranted to validate our findings in the setting of modern radiotherapy schedules and facilitate the discovery and development of metabolism-targeted agents to protect normal tissue from RT-induced EASRs and improve the quality of life in breast cancer patients undergoing RT.

Our study findings contribute to an increased understanding of metabolic pathways and biomarkers in the development of RT-induced EASRs. Metabolomic profiling is relatively quick, non-invasive, and inexpensive and supplies high-throughput methods to analyze gross patient phenotypes indicative of upstream biological processes. To the best of our knowledge, this is the first study to evaluate global metabolomics in RT-induced EASRs and explore potential targets for interventions with glutaminase inhibitors.

Conclusions

In the present study of breast cancer patients receiving adjuvant RT, alanine, aspartate, and glutamate metabolism was a key pathway of interest in predicting RT-induced EASRs given its high impact value (0.60) and significant FDR-adjusted p-value (p = 0.0028). Our findings suggest that glutaminase inhibitors may have potential applications in preventing RT-induced EASRs.

Data Sharing Statement

Data and supporting materials are not publicly available but may be available from the corresponding author upon reasonable request and approval of the Cancer Prevention Study 3 investigators.

Ethics Approval and Consent to Participate

The study was approved by the University of Miami's Institutional Review Board and written informed consent was obtained from each participant after providing a detailed description of the protocol in English or Spanish. The study complies with the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or all these areas; took part in drafting, revising, or critically

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