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Genetic biomarkers associated with response to palliative radiotherapy in patients with painful bone metastases

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Background: Palliative radiotherapy (RT) is effective in patients with painful bone metastases. Genetic factors may identify subgroup of patients who responded to RT. To identify DNA biomarkers associated with response to palliative RT.

Methods: Patients who received a single 8 Gy dose of RT for painful bone metastases were categorised into responders (n=36), non-responders (NR) (n=71). Saliva samples were sequenced to identify single-nucleotide variants (SNVs) in genes with known disease-causing variants from inflammation, radiation response, and DNA damage pathways. In univariate analysis, Cochran-Armitage trend tests were used to identify SNVs that associated with pain response (P<0.005), and the Penalized LASSO method with minimum Bayesian Information Criterion was used to identify multi-SNVs that jointly predict pain response to RT. The corresponding estimated effect of the multi-SNVs were used to drive the prognostic score for each patient. Based on it, patients were divided into 3 equal size risk groups.

Results: Forty-one significant variants were identified in univariate analysis. Multivariable analysis selected 14 variants to generate prognostic scores, adjusting for gender and primary cancer site. Eighty-nine percent of patients in the high prognostic group responded to palliative radiation therapy (P=0.0001). Estimated effect sizes of the variants ranged from 0.108–2.551. The most statistically significant variant was a deletion at position 111992032 in the *ataxin* gene *ATXN2* (P=0.0001). Five variants were non-synonymous, including *AOAH* rs7986 (P=0.0017), *ZAN* rs539445 (P=0.00078) and rs542137 (P=0.00078), *RAG1* rs3740955 (P=0.0014), and *GBGT1* rs75765336 (P=0.0026).

Conclusions: SNVs involved in mechanisms including DNA repair, inflammation, cellular adhesion, and cell signalling have significant associations with radiation response. SNVs with predictive power may stratify patient populations according to likelihood of responding to treatment, therefore enabling more efficient identification of beneficial strategies for pain management and improved resource utilisation.

Keywords: Genetic biomarker; single nucleotide variant (SNV); palliative radiotherapy (RT)

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Introduction

Cancer is one of the most prevalent causes of morbidity in Canada, with an estimated 202,400 new cases every year (1). Many cancer patients present with painful bone metastases, which contribute to a reduced quality of life (QOL) and a reduced ability to perform activities of daily living (ADL). When left unmanaged, bone metastases may lead to complications known as skeletal-related events (SREs), such as fractures and spinal cord compression that cause severe pain and functional impairment (2). As cancer treatments have improved the overall survival of cancer patients, patients are dealing with bone metastases on a long term basis (3). For example, 95% of breast and prostate cancer patients survive for at least 5 years or more after diagnosis (4). Therefore, effective pain management of bone metastases in improving QOL and restoring functional independence is an increasingly relevant aspect in cancer palliation.

Palliative radiotherapy (RT) is often used in conjunction with analgesics, hormone treatments, and bone-modifying agents to manage pain secondary to bone metastases (2). However, patients vary in their responses to palliative RT in terms of pain reduction. As defined by the International Bone Metastases Consensus Working Party, responses to palliative RT while considering analgesic dose is categorized into complete response [(CR) complete reduction in pain], partial response [(PR) partial reduction in pain], or pain progression [(PP) increase in pain] (5). Overall, 58–59% of patients have at least a partial pain reduction (CR or PR) (6). Therefore, almost half of patients would not respond to palliative RT and would have either consistent or increased pain levels.

Development of large scale genomic and sequencing technologies have enabled numerous studies to identify and validate genetic biomarkers, which are germline genetic variations in the population that are associated with a biological outcome. Single-nucleotide variants (SNVs) comprise the majority of genetic variation among individuals, and are differences at a single location in DNA (7). While several studies exist on genomic markers predictive of curative radiation treatment or radiation toxicity, genetic biomarkers have not been investigated in a palliative setting (8-11). Therefore, we aimed to identify genetic polymorphisms associated with palliative RT response in patients. These findings may be significant in enabling effective pain management strategies, as well as providing insight into the mechanism of the pain response to RT.

Methods

Patient population

Informed, written consent was obtained for cancer patients across 23 Canadian cancer centres receiving palliative RT of a single 8 Gy dose for painful bone metastases were enrolled in the randomized, double-blind placebo-controlled trial NCIC Clinical Trials Group (NCIC CTG) Symptom Control 23 (SC.23) study (12). This study was approved by the Ontario Cancer Research Ethics Board (OCREB) (No. 10-094).

Data collection

Patients were asked to fill out both the brief pain inventory (BPI) in which they reported their worst pain scores on a scale of 0–10 as well as their opioid analgesic intake on day one of RT, every day for 10 days post-RT, and at day 42 post-RT. Change in pain response from day 1 until day 42 were used to classify RT response based on definitions from the International Bone Metastases Consensus Working Party (5). RT responders consisted of patients who had CR or PR, while poor responders consisted of patients who were non-responders (NR), had PP, or stable pain (SD). Response to RT at week 6 evaluation, CR/PR as responders coded as 1, others coded as 0.

Genomic analysis

Saliva samples were obtained from patients at day of RT. The samples underwent next-generation sequencing using the Illumina TruSight^{TM} One Panel to identify SNVs in 4,813 genes with known disease-causing variants. Raw data from Illumina's MiSeq platform hg19 was mapped to a reference genome using BWA (13). Base quality score recalibration, indel realignment, duplicate removal, and variant calling using GATK (14) were performed in accordance with the principles outlined in GATK Best Practices (15). Variants with functional and clinical information were annotated using ANNOVAR to assist in subsequent variant filtering and analysis (16).

Variant selection and statistical analysis

Variants were selected from genes identified to be part of inflammatory, immune response, radiation response, or DNA damage. Associations between variants and response to RT were tested for statistical significance using the

Table 1 Baseline characteristics

Characteristic	Values
Age [years]	72 [59–78]
Sex	
Male	63 (58.9)
Female	44 (41.1)
Primary cancer site	
Prostate	34 (31.8)
Breast	26 (24.3)
Lung	24 (22.4)
Other or unknown	23 (21.5)
Karnofsky performance status	
40–60	34 (31.8)
70–80	65 (60.7)
90–100	7 (6.5)
Worst pain score at baseline	
1–4	18 (16.8)
5–6	28 (26.2)
7–10	61 (57.0)
Index site of radiated bone lesion	
Pelvis, hips, or lower limbs	43 (40.2)
Ribs, clavicle or sternum	26 (24.3)
Lumbo-sacral spine	23 (21.5)
Cervical-thoracic spine	13 (12.1)
Humerus	2 (1.9)
Response to radiation therapy	
Non-responders	71 (66.4)
Responders	36 (33.6)

Data are shown as number (percentage) or median [interquartile range].

Cochran-Armitage trend test to produce a univariate model. Significant variants (P<0.005) underwent penalized variable selection to identify a multi-SNVs model predictive of radiation response. The SAS procedure *hpgenselect* was used with the LASSO method of variable selection using the minimum Bayesian Information Criterion. Each SNVs was coded as 0 if patients had a genotype of AA, 1 for AB and 2 for BB. Patients' prognostic scores for response to RT at 6 weeks were derived from the sum of the estimate of effect in the *hpgenselect* model of each of SNVs in the multivariable model, multiplied by the corresponding SNV value (0, 1 or 2). The prognostic score of response to RT at 6-weeks was used to divide patients into three groups: low (<1/3 quantiles), coded as 0 *vs.* middle (≥1/3 quantiles but <2/3 quantiles), coded as 1 *vs.* high (≥2/3 quantiles), coded as 2. In multivariable analysis, a logistic regression model was produced with response status as the dependent outcome, and the risk groups model adjusted for gender and primary cancer site as the independent factor.

Pathway analysis was conducted for significant variants and their associated genes to identify genes in commonly reoccurring biological pathways implicated in radiation response. A literature search of significant variants was also conducted and independently analysed for reproducibility of significance.

Results

Baseline demographic and clinical characteristics of the patient population in this study are shown in *Table 1*. The median age of patients was 72, with the inter-quartile range of 59 to 78 years old. Females represented 41.1% of patients. Prostate was the most common site of primary cancer (31.8%), followed by breast (24.3%) and lung (22.4%). The most common Karnofsky performance status was between 70–80, and the most common worst pain score at baseline was between 7–10. The most common location of radiation treatment was to the pelvis, hips, or lower limbs (40.2%), followed by the ribs, clavicle or sternum (24.3%), then the lumbo-sacral spine (21.5%). Out of 79 patients included in this study, 36 responded to palliative RT (33.6%), and 71 did not (66.4%).

Multivariable model

Sequencing of 4,813 genes found 41 variants significantly associated with palliative radiation response in univariate analysis (*Table S1*). The multivariable model selected 14 SNVs (*Table 2*). A high prognostic score corresponded with a higher chance of response to RT. Univariate analysis of the risk group by response status using the Chi-squared tests showed that 89% of patients in high prognostic group responded to RT (P<0.0001, *Table 3*).

A deletion variant at position 89986545 on chromosome 16 of the gene *MC1R* had the largest effect size (2.55). *MC1R* produced a melanocyte-stimulating hormone

Table 2 Genetic variants associated with response to palliative radiation therapy

Gene	Chr	Position	dbSNP ID	R:A	Protein change	Responder SNV: 0, 1, 2	Non-responder SNV: 0, 1, 2	ExAC	Function	P	Effect size
CPT1A	Chr11	68560780	rs75677837	C:T	Intron variant	30, 5, 1	70, 1, 0	0.0374	Fatty acid metabolism	0.0029	1.49
MTTP	Chr4	100510859	rs991811	T:C	Synonymous	10, 12, 14	37, 25, 9	0.4036	Lipoprotein assembly	0.0015	0.31
AOAH	Chr7	36552656	rs7986	G:A	p.Pro684Leu at 3'UTR	10, 19, 7	40, 27, 4	0.3883	Inflammatory response	0.0017	1.04
ZAN	Chr7	100373367	rs539445	G:C	p.Ser2035Thr	10, 12, 14	36, 28, 7	0.4251	Cellular adhesion	0.0008	0.11
CYP2G1P	Chr19	41406411	rs17726493	C:T	Not available	19, 14, 3	59, 10, 2	Not available	Cytochrome pseudogene	0.0017	0.71
ATXN2	Chr12	111992032	Not available	A:–	Intron variant	26, 10, 0	69, 2, 0	Not available	Endocytosis, neurodegenerative disorders	0.0001	1.96
IFRD1	Chr7	112102355	rs2253962	T:G	Synonymous	13, 19, 4	49, 18. 4	0.2378	Cell differentiation	0.0030	0.76
PTPRJ	Chr11	48145166	rs2270993*	G:A	Synonymous	21, 15, 0	60, 11, 0	0.1403	Cell signalling	0.0029	0.31
MC1R	Chr16	89986545	Not available	C:–	Synonymous	29, 7, 0	70, 1, 0	Not available	Melanocyte-stimulating hormone receptor	0.0008	2.55
RGR	Chr10	86012713	rs1042454*	C:T	Synonymous	8, 18, 10	32, 33, 6	0.4184	Vision	0.0027	0.78
ZAN	Chr7	100373077	rs542137	C:G	p.Phe1969Leu	10, 12, 14	36, 28, 7	0.4252	Cellular adhesion	0.0008	0.11
GCGR	Chr17	79770740	rs5384	C:T	Synonymous	20, 14, 2	60, 10, 1	0.1177	Glucagon receptor	0.0014	1.08
RAG1	Chr11	36595600	rs3740955	A:G	p.His249Arg	7 19 10	37 25 9	0.447	Lymphocyte development	0.0014	0.53
GBGT1	Chr9	136029301	rs75765336	C:T	p.Ala230Thr	30, 6, 0	70, 1, 0	0.0041	ABO blood group	0.0026	2.26

Gene, name of gene harbouring SNV; Chr, chromosome of variant; Position, chromosomal location of variant; dbSNP ID, SNV identification (*, variant with published clinical associations); R:A, reference allele and alternative allele; Protein change, change of amino acid; Responder SNV and Non-responder SNV, number of individuals with 0, 1, or 2 copies of the alternative allele; ExAC, population frequency of alternative allele from Exome Aggregation Consortium; Function, biological function of gene; P, significance found in univariate analysis, as determined by the Cochran-Armitage trend test; Effect size, estimate of effect on predicting response to radiotherapy. 3'UTR, 3' untranslated region; SNV, single-nucleotide variant.

Table 3 Prognostic risk group by response status

Response status	Prognostic groups, n (%)			
	Low (N=35)	Middle (N=36)	High (N=36)	Total (N=107)
Non-responders	33 (94.3)	34 (94.4)	4 (11.1)	71 (66.4)
Responders	2 (5.7)	2 (5.6)	32 (88.9)	36 (33.6)

receptor. The variant with the highest statistical significance was from an intronic variant of a base deletion at position 111992032 of chromosome 12, corresponding to the gene *ataxin* (*ATXN2*, P=0.0001). Our model identified two variants that belonged to a single gene, the cell adhesion gene *ZAN*. These were the rs539445 variant, which produces an amino acid change at position 2,035 from serine to threonine, and the rs542137 variant, which produces an amino acid change at position 1,969 from phenylalanine to leucine.

Two variants have published associations. The rs2270993 G973A synonymous variant of the cell signalling gene *PTPJR* was found by Aya-Bonilla *et al.* to be associated with susceptibility to non-Hodgkin's lymphoma (17). *PTPJC* produces a tyrosine phosphatase receptor involved in signal transduction of MAPK signalling of cell growth, proliferation, and angiogenesis, and is putative tumour-suppressor gene. The rs1042454 C:T synonymous variant of the retinal G-protein coupled receptor gene *RGR* was investigated in two studies. Singh *et al.* found that it is associated with autosomal recessive retinitis pigmentosa (18). Grupe *et al.* investigated the association of chromosome 10 variants and late-onset Alzheimer disease (19). However, the *RGR* variant rs1042454 was not found to be significant.

Discussion

Radiogenomic studies have established that SNV from genes in the pathways of oxidative stress, inflammation, DNA damage signalling and repair, and cell cycle control are involved in determining an individual's sensitivity to radiation (20). In radiation therapy, ionizing radiation causes double-strand breaks in the DNA, leading to downstream repair mechanisms and signals to the cell to stop cell cycle progression or to undergo apoptosis. Since cancer cells have greater genomic instability, radiation can cause DNA damage to an irreparable degree in these cells, leading to tumour shrinkage. In the palliative setting, radiation can reduce pain through shrinking tumours or metastases that compress nerves or activate pain transducing afferent neurons (21). Therefore, response to palliative RT also involves cytotoxic response to DNA damage in tumour cells.

Our study identified a variant, rs3740955 from the from the *RAG1* gene corresponding to a change from histidine to arginine at position 249, that is implicated in response to palliative RT. RAG1 is part of the machinery that produces DNA breaks that facilitates VDJ recombination critical in lymphocyte development (22). A study by Gee *et al.* on patients with breast cancer who had adjuvant radiation therapy after breast-conserving surgery identified markers associated with radiation response as measured by local recurrence and survival (23). The authors found that low expression of *RAG1* was significantly associated with local recurrence in multivariate analysis. Genetic variation of *RAG1* may influence response to radiation, and confer differences in susceptibility of tumour cells to radiation damage, therefore producing differences in the ability of radiation to reduce pain through causing tumour shrinkage.

Bone metastases cause bone pain through a combination of nociceptor stimulation by growth factors and proinflammatory molecules produced from the tumour environment, and nerve injury from the growing tumour (24). These may be identified as changes in cellular signalling cascades. Our study identified several variants of intracellular and intercellular signalling that may be implicated in the pain response to radiation therapy. This includes the rs2270993 variant in *PTPRJ*, a tumour suppressor gene with antiproliferative functions through inhibiting cell growth, migration, and vascularization (25). *PTPRJ* has also been implicated in several cancer types, including colorectal cancer, non-Hodgkin's lymphoma, and esophageal squamous cell carcinoma (17,26,27). Another variant that is part of a signalling gene is the deletion at position 89986545 of *MC1R*. This gene is involved in the development of melanocytes and in sensitivity to solar UV radiation damage (28). Epidemiological studies have also found an association of *MCR1* variants with risk of skin cancer.

There are currently several options in managing cancer pain, including radiation, non-steroidal anti-inflammatory drugs, opioids, systemic radioisotopes, corticosteroids, antidepressants, and bisphosphonates. The potential ability of genetic biomarkers to identify patients who respond to palliative RT would allow targeted options for end-of-life care. In the palliative setting, predicting whether a patient will respond to RT would not only allow more

efficient management strategies, but also reduce the fatigue and psychological burden from unnecessary treatments that do not produce the desired effect. In addition, it would free-up healthcare resources through serving those patients identified as likely candidates to benefit from RT. Therefore, further research should be conducted to validate the present multi-SNV model. The identification of genetic biomarkers that stratify patients into responders and NR of palliative RT is of great clinical utility to patients and to healthcare management.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: This study was approved by the Ontario Cancer Research Ethics Board (OCREB) (No. 10-094) and written informed consent was obtained from all patients.

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Supplementary

Table S1 Significant variants identified in univariate analysis (P<0.005)

Gene	Chromosome	Location	Genetic change	Responder SNV	Non-responder SNV	P
*CPT1A**	Chr11	68560780	C:T	30, 5, 1	70, 1, 0	0.0029
*MTTP**	Chr4	100510859	T:C	10, 12, 14	37, 25, 9	0.0015
*AOAH**	Chr7	36552656	G:A	10, 19, 7	40, 27, 4	0.0017
*ZAN**	Chr7	100373367	G:C	10, 12, 14	36, 28, 7	0.0008
*CYP2G1P**	Chr19	41406411	C:T	19, 14, 3	59, 10, 2	0.0017
*ATXN2**	Chr12	111992032	A:–	26, 10, 0	69, 2, 0	0.0001
*IFRD1**	Chr7	112102355	T:G	13, 19, 4	49, 18, 4	0.0030
*PTPRJ**	Chr11	48145166	G:A	21, 15, 0	60, 11, 0	0.0029
*MC1R**	Chr16	89986545	C:–	29, 7, 0	70, 1, 0	0.0008
*RGR**	Chr10	86012713	C:T	8, 18, 10	32, 33, 6	0.0027
*ZAN**	Chr7	100373077	C:G	10, 12, 14	36, 28, 7	0.0008
*GCGR**	Chr17	79770740	C:T	20, 14, 2	60, 10, 1	0.0014
*RAG1**	Chr11	36595600	A:G	7, 19, 10	37, 25, 9	0.0014
*GBGT1**	Chr9	136029301	C:T	30, 6, 0	70, 1, 0	0.0026
GP6	Chr19	5552559	T:C	3, 4, 29	17, 18, 36	0.0047
MTUS1	Chr8	1761269	T:C	32, 4, 0	71, 0, 0	0.0042
TET1	Chr10	70332580	A:G	9, 21, 6	43, 20, 8	0.0043
CASR	Chr3	122003769	A:G	21, 9, 6	59, 9, 3	0.0037
ALDH4A1	Chr1	19202896	G:A	9, 15, 12	33, 30, 8	0.0037
DCLK1	Chr13	36402426	A:G	8, 17, 11	33, 29, 9	0.0047
FLNB	Chr3	58134505	A:G	32, 4, 0	71, 0, 0	0.0042
TAS2R19	Chr12	11174942	C:T	30, 6, 0	70, 1, 0	0.0026
XIAP	Chrx	123044718A	AA:–	32, 4, 0	71, 0, 0	0.0042
ST14	Chr11	130058437	G:A	32, 4, 0	71, 0, 0	0.0042
SARDH	Chr9	136555629	T:C	8, 17, 11	36, 25, 10	0.0034
MADD	Chr11	47312374	A:G	17, 13, 6	53, 14, 4	0.0043
F2	Chr11	46745003	C:T	15, 17, 4	54, 12, 5	0.0035
FLT3	Chr13	28624294	G:A	5, 9, 22	19, 31, 21	0.0049
SPP1	Chr4	88902692	T:C	12, 16, 8	42, 24, 5	0.0038
MDN1	Chr6	90390443	C:A	5, 17, 14	26, 32, 13	0.0040
GP6	Chr19	55525894	G:A	3, 4, 29	17, 18, 36	0.0047
NR1H3	Chr11	47282024	C:T	15, 15, 6	55, 12, 4	0.0005
TET1	Chr10	70405855	A:G	2, 6, 28	12, 25, 34	0.0047
DLGAP2	Chr8	1616640	G:A	31, 5, 0	43, 21, 7	0.0042
MUC5B	Chr11	1260145	T:C	8, 28, 0	37, 34, 0	0.0031

Table S1 (*continued*)

Table S1 (*continued*)

Gene	Chromosome	Location	Genetic change	Responder SNV	Non-responder SNV	P
SPP1	Chr4	88903853	C:T	12, 16, 8	42, 24, 5	0.0038
NAV2	Chr11	19914118	A:C	32, 4, 0	71, 0, 0	0.0042
SLCO1B1	Chr12	21331625	C:T	8, 21, 7	33, 34, 4	0.0036
TAS2R19	Chr12	11174952	A:T	30, 6, 0	70, 1, 0	0.0026
MDN1	Chr6	90402482	G:A	5, 17, 14	26, 32, 13	0.0040
POLR3B	Chr12	106838340	T:C	11, 15, 10	39, 25, 7	0.0046

Gene, genetic symbol of gene housing variant (*, variants selected in multi-SNV model). Chr, chromosome of variant; Location, chromosomal location of variant; Genetic change, reference allele and alternative allele; Responder and Non-responder, number of individuals with 0, 1, or 2 copies of the alternative allele; P, significance found in univariate analysis, as determined by the Cochran-Armitage trend test. SNV, single-nucleotide variant.