Changes in Whole Blood Gene Expression after Computed Tomography in Children: A Pilot Study

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ABSTRACT: Computed tomography (CT) exposes patients to ionizing X-irradiation (IR) that can alter the expression of genes responsible for controlling complex regulatory pathways. We sought to determine trends in the expression of 24 documented radiation-responsive genes linked to cancer in vivo. A total of 17 children (0.25–6 years old) undergoing medically indicated CT examinations with radiation doses ranging from 92.46 to 525.55 mGy cm (equivalent to effective doses of 0.78–11.30 mSv) were enrolled. Blood was drawn immediately before and 1 hour after their CT exams and mixed with an RNA additive for stabilization of gene expression. RNA samples of 14 of the 17 children were analyzed on a gene expression microarray. Absolute changes in gene expression were subtle, averaging less than 10%, but trends in expression changes of several genes were observed. ERP29, an endoplasmic reticulum chaperone, thought to be involved in the folding of secretory proteins, showed significant change in expression (8% decrease, $P = 0.002$) in the expected direction consistent with previous literature. PCNA expression increased linearly with CT dose (mGy cm) ($P = 0.001$). TP53 and FLT3LG expression increased linearly with effective dose (mSv) ($P = 0.02$ and $P = 0.02$). Previous IR exposure was associated with decreased GADD45A ($P = 0.001$) and FLT3LG ($P = 0.03$) and increased MDM2 expression ($P = 0.02$). We observed in this pilot study modest gene expression changes in the 24 IR-responsive candidate genes studied. Our results showed trends in gene expression changes, and they need to be confirmed in future studies with larger sample sizes to help develop risk assessments and preventive modalities for young patients undergoing CT.

KEYWORDS: X-irradiation, children, gene expression, CT scan

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Introduction

Computed tomography (CT) is an extremely useful imaging modality in modern medicine because it allows rapid, painless, and accurate diagnosis of most organ system pathologies within seconds. The application of CT, however, involves significant exposure to ionizing X-irradiation (IR) to produce tomographic slices that can be used for diagnostic purposes.

CT use has risen substantially over the past few decades, particularly in children.[1] Between 1995 and 2008, the number of CT scans performed in the pediatric emergency department (ED) increased five-fold, while the number of ED visits during the same time frame did not change.[2] This increase has been attributed largely to technological advances in CT such as clearer images and faster image acquisition times, which have improved CT-based diagnosis substantially.[3] The extremely fast scan times have nearly obviated the need for sedation when obtaining a pediatric CT, which has been especially beneficial for physicians dealing with very young, very sick, and/or non-cooperative children.[4,5] While some investigators expected the proportion of CT examinations in the pediatric population to continually increase,[1,3,6,7] a recent report has suggested otherwise.[8] However, even with the slowdown in CT rise,[9] the incidence of pediatric CT examinations is at present still very high.

IR from CT scans has been documented to elicit various detrimental cellular responses because of free radical reactions. Such reactions have been linked to DNA lesions, base damage, and cross-linking of proteins, all of which can ultimately increase the risk of developing cancer.[1,10] These risks are more prominent in children than adults because of their higher radiosensitivity and longer life expectancy to have cumulative exposure.[7,11] Two recently published large epidemiological studies assessing IR and cancer risk in children and young adults exposed to medically indicated CT scans reported that a cumulative dose of 50–60 mGy received from CT scans could triple the risk of developing leukemia and brain cancers[12] and that cancer incidence was 24% greater in those exposed to CT scans than those not exposed.[13]

Furthermore, general (non-pediatric focused) hospitals are less likely to use pediatric-specific radiation-reduction protocols and instead use techniques that are likely to result in children being exposed to adult-size radiation doses, which are

significantly higher than those used for children.[14] This is of great concern in light of a recent report on imaging frequency that estimated 89.4% of pediatric CT scans performed in the ED were done at primary adult facilities.[2]

Current estimates suggest that CT scans lead to a lifetime cancer risk of up to 2%, with a higher risk for young children.[1] In addition, some investigators have found that intellectual development may be adversely and permanently affected in children receiving head IR.[15] A radiation risk assessment tool based on methods of biologic effects of ionizing radiation (BEIR) VII has also been developed to help estimate lifetime attributable risk for various radiation-related cancer types.[16] IR has also been shown to alter the expression of genes responsible for controlling complex regulatory pathways, including cell cycle, apoptosis, and DNA repair.[17]

In particular, the following 24 genes have been identified as radiation-sensitive markers in blood cells: GADD45A, CDKN1A (p21), ATM, ERP29, TP53, CDKN2A (p16), MUC1, CDH6, DDB2, XPC, DR5, FHL2, CCNG1, PCNA, CCNB1, MDM2, BAX, MAPK8 (JNK1), ALB, CPI (KNG1), FTL3LG, HP, RPA2, and NFKB. These genes were chosen mostly from the literature between years 2000 and 2006.[18–37]

Owing to the wide variety of experimental details (eg, radiation doses, radiation type, in vitro vs. in vivo systems) in the abundance of publications relating to gene expression effects from IR, we focused on review articles that condensed essential information on the effects of IR on various tissue (including hematological) types, which yielded 24 genes that were most likely to exhibit change in blood cells after IR exposure and were linked to cancer.

In this pilot study, we tested whether the expression of these 24 radiation-sensitive markers changed in vivo in stabilized whole blood of young children after medically indicated CT examinations involving relatively low IR doses. To our knowledge, this is the first in vivo study in children (unlike previous reports in adults) to examine the effect of IR from medically indicated CT examinations on gene expression changes in radiation-responsive genes.

Methods

Patient recruitment. In all, 17 pediatric patients (0.25–6 years old) undergoing medically indicated CT scans were enrolled in the ED at Kapi'olani Medical Center for Women and Children in Honolulu, HI, with signed consent from their parent or legal guardian. Exclusion criteria included children with immediate risk of decompensation, children weighing less than 9 lbs, and children with complex medical problems such as cancer. Information regarding the child's age, birth history, past medical history, medication use, ethnicity, overall health condition, allergies, height (in meters) and weight (in kilogram), vitamin intake, and a detailed radiological history was obtained from interviews with the parent or legal guardian and also through retrieval of hospital records. Blood draw times, CT scan times, and CT doses (in DPLC/mSv) were also documented. This pilot study has been approved by the Western Institutional Review Board and the University of Hawaii Committee on Human Studies, and has been performed in accordance with the ethical standards and principles laid down in the 1964 Declaration of Helsinki and its later amendments.

CT parameters. CT examinations were performed using a LightSpeed VCT Select 64 slice CT scanner. The radiation dose is expressed in gray (Gy) or sievert (Sv) units, both expressed in SI units as J/kg.

In all, 1 Gy is the absorption of 1 J of energy in the form of IR per kilogram of matter, eg, human tissue. The weighting factor converts this into equivalent dose (Sv) used for whole body exposure, which is dependent on the type of radiation (in the case of X-rays, this factor is 1.0). This unit gets converted by tissue weighting factors into the effective dose (Sv), which is dependent on the type of tissue. The CT used for our subjects had doses ranging from 92.46 to 525.55 mGy cm, equivalent to effective doses of 0.78–11.30 mSv.

The radiation history of each child was expressed as dose in relative numbers according to published data of regulatory and other agencies: each head, chest, and abdominal or pelvic CT was accordingly assigned 1.0, 4.0, and 5.0 units, respectively, and each X-ray, 0.01 units for a posterior–anterior chest x-ray, 0.02 units for a lateral chest X-ray, and 0.35 units for an abdominal or a pelvic X-ray.[38–40]

Sample collection and processing. Peripheral whole blood (0.3–2.5 mL) was drawn by venipuncture and mixed with the PAXgene™ RNA additive (PreAnalytiX GmbH) in the manufacturer's recommended proportions for each child immediately before (pre-CT) and 1 hour after (post-CT) their scheduled CT examinations. When possible, EMLA cream was used to minimize pain during venipuncture. If a normal saline IV lock was in place for medical reasons, ca. 2.5 mL of blood was withdrawn and then discarded before obtaining blood for the study. After the CT examinations, all tubes were transported immediately to the University of Hawaii Cancer Center laboratory in a sealed leak proof bag in a biohazard cooler at room temperature and protected from light. Upon arrival, PAXgene™ tubes were incubated at room temperature overnight followed by freezing at –20°C for 4–10 hours and finally long-term storage at –80°C until RNA extraction.

Isolation of total RNA from whole blood. Intracellular RNA was extracted using the PAXgene™ RNA extraction kit per manufacturer's instructions (PreAnalytix GmbH). Total extracted RNA was quantified using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific), and RNA quality was monitored using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.) with RIN (RNA Integrity Number) measurements averaging 7.8 (range 6.3–8.8). RNA yields were 1–10 g. Samples with RINs below 7 were considered of poor quality and excluded from analysis. After the quality check, 1 µg of RNA was subjected to globin mRNA

removal using the Ambion GLOBINclear kit (Life Technologies), and 75–100 ng of the total RNA was converted to sense strand DNA using Ambion WT Expression Kit according to the Affymetrix WT Sense Target Labeling Assay protocol (Affymetrix Inc.). The pre-CT and post-CT samples for each child were processed together to minimize batch effects.

Microarray hybridization. Terminally biotin-labeled and fragmented DNA was hybridized to the Human Gene 1.0 ST arrays (Affymetrix, Inc.) for 16 hours. Following hybridization, the arrays were washed and stained, and the fluorescent signals on the arrays were scanned on the Affymetrix TG Plus scanner to produce an image file of the chip. With one exception, the pre-CT and post-CT arrays for each child were processed in the same batch. The lowest *P*-value across the study scan date batch effect for the 24 genes was 0.13. Overall results did not change after removing the one sample pair that was not processed in the same batch.

Statistical analysis. The fluorescence intensities measured on the scanned images were quantified and quantile normalized using the Partek Genomics Suite analysis platform. Analyses of the differentially expressed genes were performed using a paired sample *t*-test. Data were analyzed using the REG procedure in the SAS 9.3 software. Stepwise multiple regression with the forward entry method was used. The significance level for entry into the model was 0.05. Two separate analyses were run. In the first analysis, the outcome variable was the mean pre- to post-CT change (or delta) and the possible predictors were baseline (pre) values, CT dose, effective dose, radiation history, general health, multivitamin use, contrast, CT type, sex, age, and BMI. In the second analysis, the outcome variable was the mean baseline (pre) value and the possible predictors were radiation history, multivitamin use, and contrast use.

Results

In this study, blood samples from 17 children were obtained immediately before and 1 hour after undergoing medically indicated CT scans. Characteristics of the participants are presented in Table 1. The children ranged in age from 3 months to 6 years. A total of 12 children received CT scans of the head region, while the remaining children received CT scans of the abdomen ($n = 3$), neck ($n = 1$), or chest ($n = 1$) region. The CT and effective doses ranged from 92.46 to 525.55 mGy cm, equivalent to 0.78–11.30 mSv, respectively.

Microarray analysis was carried out to identify changes in the expression levels of the studied 24 radiation responsive genes. The IR-induced expression changes for these 24 genes from previous and the current study are shown in Table 2. For each gene in the current study, pre- and post-CT pairwise comparisons were performed for 14 of the 17 children after excluding RNA samples that did not produce sufficient cRNA to hybridize onto a chip (pre-CT sample of patient 7) or whose RINs were <7 (pre- and post-CT samples of patients 10 and 11), which indicated degraded RNA.

Table 1. Demographics of study participants.

PATIENT ID	GENDER	HEIGHT (cm)	WEIGHT (kg)	AGE (Y)	CT DOSE (mGy-cm)	EFFECTIVE DOSE (mSv)	CT LOCATION	CT TYPE	CONTRAST USE	MULTIVITAMIN INTAKE	RADIATION HISTORY (DOSE IN RELATIVE NUMBERS)
1	M	88.2	11.9	2.0	372.85	2.50	Head	Axial	No	Yes	0.02
2	M	84.0	9.8	1.2	372.85	2.50	Head	Axial	No	No	0.50
3	M	99.0	14.5	2.0	376.58	11.30	Abdomen	Helical	Yes	No	1.02
4	M	95.0	13.9	3.0	147.37	4.42	Abdomen-Pelvis	Helical	Yes	No	0.01
5	M	118.0	28.0	5.0	104.13	2.08	Abdomen-Pelvis	Helical	Yes	No	0.05
6	F	96.0	14.0	3.0	340.89	2.28	Head	Axial	No	Yes	0.02
7	M	65.0	12.0	1.4	310.71	2.08	Head	Axial	No	No	2.53
8	M	123.0	40.6	6.0	355.10	1.42	Orbitis	Axial	Yes	No	2.05
9	F	118.0	20.0	5.0	195.30	0.78	Orbitis	Axial	Yes	Yes	0.00
10	F	102.0	14.7	4.0	426.12	2.86	Head	Axial	No	Yes	4.37
11	M	105.0	15.5	4.0	426.12	2.86	Head	Axial	No	Yes	0.02
12	M	102.0	18.6	6.0	525.55	2.10	Head	Axial	No	No	14.12
13	M	112.0	19.0	4.0	236.14	6.14	Mastoid bone	Axial	Yes	No	1.00
14	F	114.0	18.7	4.0	106.48	1.28	Chest	Helical	Yes	No	0.05
15	M	65.0	7.1	0.3	92.46	1.57	Neck	Helical	Yes	No	0.03
16	M	92.0	12.1	1.8	426.12	2.86	Head	Axial	No	Yes	1.01
17	M	68.0	11.2	1.3	340.89	2.28	Head	Axial	No	Yes	0.00

Table 2. Twenty-four Candidate Ionizing Radiation Responsive Genes. Data from previous studies and the current study in children.

GENE	GENE NAME	GENE ARRAY TRANSCRIPT ID	RELEVANCE TO CANCER	OBSERVED IN PREVIOUS STUDIES					OBSERVED IN THIS STUDY		
				DOSE (GAMMA OR X-RAYS)	DETECTION TIME FROM EXPOSURE	ANALYTE	REFERENCE #	MAIN TREND	MAIN TREND[2]	PRE- TO POST-CT FOLD CHANGE, MEAN (MEDIAN)[3]	*P*-VALUE[4]
GADD45A	Growth arrest and DNA-damage-inducible protein (alpha)	7902227	A	2–50 cGy	0.5–48 hrs	mRNA	18,19	+	+	+1.04 (1.04)	0.24
CDKN1A (p21)	Cyclin-dependent kinase inhibitor 1A	8119088	B	2–50 cGy	0.5–48 hrs	mRNA	18	+	–	–1.02 (–1.02)	0.58
ATM	Ataxia telangiectasia mutated	7943620	C	0.1 Gy–>4 Gy	<1–24 hrs	mRNA	20,25	+	None	–1.02 (0.01)	0.65
ERP29	Endoplasmic reticulum protein 29	7958819	B	0.1 Gy–>4 Gy	<1–24 hrs	Protein	20,26	–	–	–1.08 (–1.06)	**0.002**
TP53	Tumor protein p53	8012257	B	0.1 Gy–>4 Gy	<1 hrs–>7 days	Protein	20,27	+	None	+1.01 (1.03)	0.81
CDKN2A (p16)	Cyclin-dependent kinase inhibitor 2A	8160441	B	0.1 Gy–>4 Gy	1 hrs–>7 days	Protein	20,28	+	–	–1.03 (–0.02)	0.38
MUC1[1]	Mucin 1, cell surface associated	7920642	D	0.1 Gy–>4 Gy	4 hrs–3–7 days	mRNA	20	+	+	+1.06 (1.03)	0.18
CDH6	Cadherin 6, type 2, K-cadherin (fetal kidney)	8104663	E	0.2 Gy–0.5 Gy	4 hrs	mRNA	21	+	None	–1.02 (–0.01)	0.61
DDB2	Damage-specific DNA binding protein 2, 48 kDa	7939738	B	0.2 Gy–0.5 Gy	24–48 hrs	mRNA	21,22	+	–	–1.01 (0.00)	0.81
XPC	Xeroderma pigmentosum, complementation group C	8085486	B	0.2 Gy–0.5 Gy	24–48 hrs	mRNA	21,22	+	+	+1.05 (1.05)	0.11
NFKB1	Nuclear factor of kappa light poly-peptide gene enhancer in B-cells 1	8096635	S	0.5 Gy	8 hrs	mRNA	24,30	+	None	+1.02 (1.01)	0.64
TNFRSF10B (DR5)	Tumor necrosis factor receptor superfamily, member 10 b	8149733	F	1 Gy	12 hrs	mRNA	23	+	None	–1.00 (–1.03)	0.91
FHL2	Four and a half LIM domains 2	8054377	G	1 Gy	12 hrs	mRNA	23	+	+	+1.04 (1.03)	0.36
CCNG1	Cyclin G1	8109697	H	1 Gy	12 hrs	mRNA	23	+	+	+1.02 (1.07)	0.69
PCNA	Proliferating cell nuclear antigen	8064844	I	1 Gy	12 hrs	mRNA	23	+	–	–1.06 (–1.01)	0.16
CCNB1	Cyclin B1	8105828	J	1.1–>4 Gy	<1–24 hrs	mRNA	20,31	+	+	–1.01 (1.02)	0.89
MDM2	Homo sapiens Mdm2 p53 binding protein homolog (mouse)	7956989	K	1.1–>4 Gy	<1–24 hrs	mRNA	20,27	+	–	+1.02 (–1.01)	0.66
BAX	BCL2-associated X protein	8030158	L	1–>4 Gy	1–48 hrs	mRNA	20,32	+	–	–1.02 (–1.03)	0.20
MAPK8 (JNK1)	Mitogen-activated protein kinase 8	7927389	M	1–>4 Gy	1–48 hrs	Protein	20,33	+	–	–1.03 (–0.04)	0.51
ALB	Albumin	8095628	N	1.1–>4 Gy	4 hrs–3–7 days	Protein	20,34	+	–	–1.04 (–1.07)	0.33
KNG1 (CPI)	Kininogen 1	8084679	O	1.1 Gy–>4 Gy	24 hrs–3–7 days	mRNA	20,35	+	+	+1.01 (0.01)	0.76
FLT3LG	Fms-related tyrosine kinase 3 ligand	8030339	P	1.1 Gy–>4 Gy	25 hrs–>7 days	mRNA	20,37	+	None	–1.03 (–1.06)	0.45
HP	Haptoglobin	7997188	Q	1.1–>4 Gy	4–48 hrs	mRNA	20,34	+	None	–1.01 (–1.01)	0.88
RPA2	Replication protein A2, 32 kDa	7914141	R	>4 Gy	<1–24 hrs	mRNA	20,36	+	–	–1.03 (1.00)	0.31

Notes: A = Inhibits tumor initiation and progression, B = Tumor suppressor gene, C = Reduced protein leads to increased mutation rates, D = Inhibits anti-tumor immune response, E = Prognostic marker for renal cell carinoma, F = Reduced protein leads to diminished apoptosis of tumor cells, G = can act as a tumor promoter or tumor suppressor, H = associated with liver cancer, I = Marker for cancer progression and prognosis, J = Excess protein may lead to accumulation of genetic abnormalities, K = Oncogene, L = Proapoptotic tumor suppressor gene, M = Reduced protein can lead to increased tumor formation, N = Source for tumour bioenergetics and macromolecular synthesis? O = Essential role in prevention of metastasis of cancer cells, P = Can affect response to AML chemotherapy, Q = Prognostic serum biomarker for breast and ovarian cancer, R = Prognostic marker for colon cancer, S = Initiation or acceleration of tumorigenesis. [1]Although not previously measured in blood cells, MUC1 was included due to its known interaction with ATM [Ref 29]; [2]The trend observed in at least 6 children with a minimum of 10% change ("+" = up, "–" = down); [3]n = 14 paired pre- and post-CT pairs; [4]*P*-value of mean post- to pre-CT fold change.

Overall, the observed changes in pre- and post-CT whole blood of the 14 children were very modest; one gene (ERP29) showed a significant change in expression in the expected direction (downregulation, $P = 0.002$), while three other genes were altered with borderline significance; PCNA was downregulated ($P = 0.16$), while MUC1 and XPC were both upregulated ($P = 0.18$ and $P = 0.11$, respectively) (dot-plots of post- vs. pre-CT changes are shown in Fig. 1).

Most of the observed gene expression changes showed very subtle trends with absolute average fold changes less than 1.1 fold. However, interestingly, the directionality of the changes observed in children was consistent with those observed in previous ex vivo and in vitro studies for 10 of the 24 genes (Table 2). Since these modest fold changes may have stemmed from the heterogeneity in the whole blood response among the children, we attempted to identify the main trends for these expression changes. In doing so, we found five genes (GADD45A, ERP29, MDM2, MAPK8, ALB) that responded with a minimum of 10% change in expression levels between the post- and pre-CT samples in at least six children. (Dot-plot for ERP29 is shown in Fig. 1). However, only two of these five genes (GADD45A and ERP29) showed a trend in the expected direction (ie, according to previous studies; Table 2), and the fold changes among the main trend cases for the five genes were still modest—maximizing at a 1.18 absolute fold change among seven cases (MAPK8, –1.18, data not shown).

Regression analyses revealed dose-dependent increases in PCNA expression with increasing CT dose (mGy cm) ($P = 0.001$; Table 3) and dose-dependent increases in TP53 and FLT3LG expression with increasing effective dose (mSv) ($P = 0.02$ and $P = 0.02$). Previous IR exposure from CT and/or X-ray examinations was associated with decreased expression of GADD45A ($P = 0.001$) and FLT3LG ($P = 0.03$) and increased expression of MDM2 ($P = 0.02$) in post- versus pre-CT samples. Multivitamin intake was associated with decreased gene expression levels of CDH6 ($P = 0.01$) and increased gene expression levels of TNFRSF10B ($P = 0.02$), PCNA ($P = 0.000001$), and MDM2 ($P = 0.04$; Table 3).

In addition, we found that a higher incidence of previous IR exposure was correlated with higher pre-CT gene expression levels of XPC ($P = 0.047$), MDM2 ($P = 0.01$), and BAX ($P = 0.001$) and lower baseline levels of ALB ($P = 0.04$; Table 4). We also found a trend between multivitamin intake and higher baseline gene expression levels of TP53 ($P = 0.02$), BAX ($P = 0.001$), and FLT3LG ($P = 0.04$).

Discussion

IR from CT scans has been well documented to elicit a wide variety of detrimental cellular responses. IR such as X-rays are able to ionize surrounding atoms and molecules,[1] and, including humans, hydroxyl molecules are the most common targets of ionization because of the abundance of water in the body. Ionization generates highly reactive free radicals that can damage relevant biological systems and lead to DNA lesions, base damage, and protein cross-links, all of which can lead to the induction of fatal cancers.[1,10,41] CT-induced IR has also been shown in vivo to significantly decrease the plasma levels of several important fat soluble vitamins.[42]

In this pilot study, we sought to determine whether IR from CT would lead to changes in gene expression levels in young children undergoing CT scans for medically indicated reasons. We focused our study on 24 radiation responsive

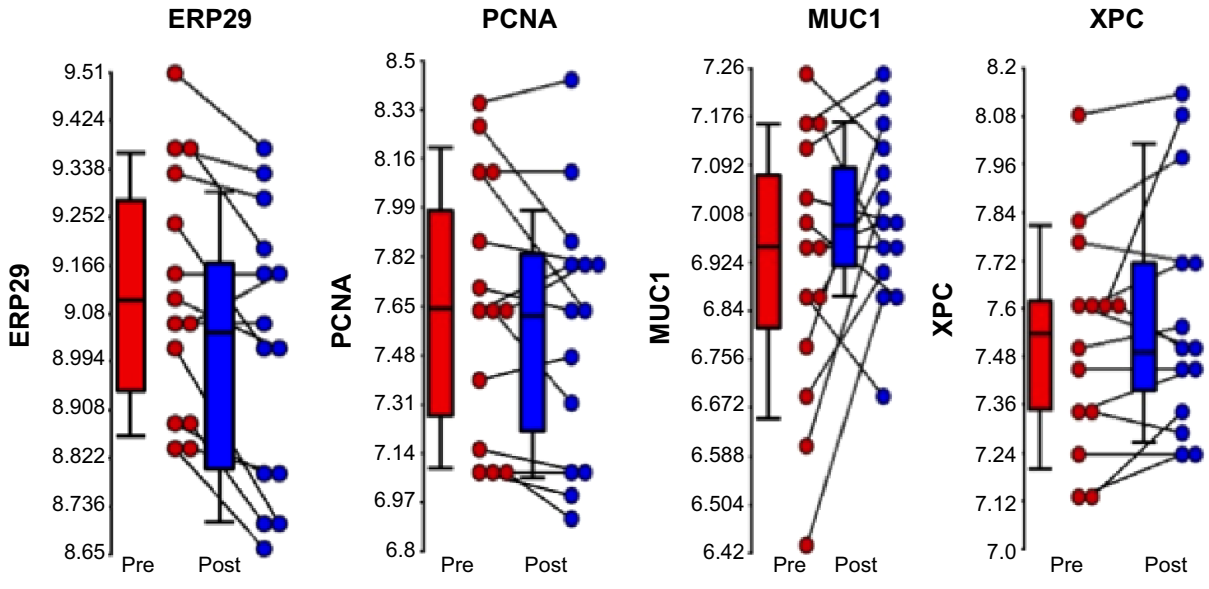

Figure 1. Dot plots showing normalized and log2 transformed pre- and post-CT gene expression levels in 14 paired samples for 4 selected affected genes. The pairs are connected with a line and the median gene expression level for each group is indicated by a line inside the box. While ERP29 was the only gene with a statistically significant change on expression in the expected direction (down-regulation, p=0.002), 3 others genes were altered with borderline significance; PCNA (down, p=0.16), MUC1 and XPC (up, p=0.18 and p=0.11, respectively).

Table 3. Post- versus pre-CT predictors of gene expression changes.

GENE	PREDICTOR	EXPRESSION LEVEL CHANGE WITH INCREASING PREDICTOR	*P*
ERP29	Age	Down	0.01
MUC1	Baseline	Down	0.001
PCNA	CT dose (mGy-cm)	Up	0.001
TP53	Effective dose (mSv)	Up	0.02
FLT3LG		Up	0.02
GADD45A	IR History	Down	0.001
FLT3LG		Down	0.03
MDM2		Up	0.002
GADD45A	General health	Down	0.02
CDH6	Multivitamin intake	Down	0.01
TNFRSF10B (DR5)		Up	0.02
PCNA		Up	0.000001
MDM2		Up	0.04

candidate genes because these genes were previously documented to be connected with functions intimately linked to cancer (Table 2).[18–37] Among the 24 genes, observations from previous studies noted post CT changes within 1 hour after radiation in 11 genes with 4 of them (ATM, ERP29, TP53, CDKN2A) showing post CT changes from very low radiation doses, as low as 0.1 Gy. Owing to their higher radiosensitivity, children are expected to react more sensitively than adults to the CT-induced radiation insult, and therefore, we included all 24 genes in our study, although some observations were reported longer than 1 hr post CT or after IR doses higher than those used in our study.[14–16,18–20]

The mRNA expression responses of the 24 candidate genes in children showed very subtle expression changes (Table 2). Only 8 of the 24 genes showed more than 1.1 fold change in expression levels between the pre- and post-CT samples and only in a subset of cases (data not shown). However, the changes were consistent with the directionality of expression change (up or down) observed in previous studies (Table 2).

Moreover, among these eight genes, only ERP29 (endoplasmic reticulum protein 29) was observed to be significantly downregulated with an average fold change of −1.08 in expression from pre- to post-CT (Table 2). Other potentially radiosensitive genes changed with borderline significance, and a larger cohort of children will be needed to confirm or reject our findings. Additionally, data from array versus RNA sequencing studies are often subject to more technical artifacts/noise, and doing a qPCR would be ideal to increase confidence of our findings. This was a limitation in our study and will certainly be considered in our future studies.

Despite these small changes, we feel the ERP29 gene deserves more attention because it has been implicated in tumorigenesis and functions as a tumor suppressor gene. It is expressed especially in secretory tissues and responds mostly to cellular stress by upregulation,[43] while both downregulation[20] and upregulation have been reported[44] in response to radiation. We did not correct for multiple test analyses for the 24 genes tested, and as always, there exists a possibility that the significant finding of ERP29 expression change was by chance. However, the ERP29 dot-plot (Fig. 1) explicitly shows a consistent decrease in expression for 11 of the 14 children, which suggests that this finding is likely not by chance.

We also examined possible factors that may predict the observed gene expression changes. Using demographic and other collected data, we regressed all variables against each of the candidate genes and found several that were correlated with changes in gene expression (Table 3). We observed dose-dependent increases in PCNA expression with increasing CT dose (mGy cm) and dose-dependent increases in TP53 and FLT3LG expression with increasing effective CT dose (mSv), which are in accordance with in vitro studies reporting increases in gene expression or protein concentrations after IR exposure.[45,46]

Next, we examined correlations between the gene expression levels and IR history. In our subjects, regression analyses revealed that previous exposure to IR (either through prior CT

Table 4. Predictors of baseline gene expression levels.

GENE	PREDICTOR	BASELINE DIRECTION WITH INCREASING PREDICTOR	*P*
XPC	IR History	Higher	0.047
MDM2		Higher	0.01
BAX		Higher	0.001
ALB		Lower	0.04
TP53	Multivitamin intake	Higher	0.02
BAX		Higher	0.001
FLT3LG		Higher	0.04

scans or X-rays) was dose dependently related to MDM2 levels. MDM2 is an oncogene and a negative regulator of p53,[47] a critical protein involved in tumor suppression through its ability to induce cell cycle arrest or apoptosis[48] under a variety of genotoxic processes, including oxidative stress.[49] In response to such stresses, p53 becomes activated and induces the expression of MDM2, which then associates with and inactivates p53, thereby creating an autoregulatory feedback loop to help moderate p53 during normal cell cycling.[48,50] Thus, in times of stress, eg, oxidative stress via IR exposure, increases in MDM2 may be anticipated. Several lines of evidence also suggest that MDM2 may have a role in tumorigenesis through a p53-independent mechanism.[51]

The inverse relationship observed between IR history and GADD45a and FLT3LG levels could be because of the varying functions of these genes. GADD45A has been reported to have the dual and seemingly conflicting functions of tumor promotion and tumor suppression with the outcome being highly dependent on the oncogenic stimuli.[52] Moderate DNA damage can trigger GADD45A to exert an antiapoptotic function, binding to other proteins to enhance DNA repair, whereas excessive DNA damage may act as a prompt for apoptosis, possibly acting as a survival mechanism during situations where irreparable cell damage is evident.[53] FLT3LG is a critical transmembrane protein well documented to stimulate the proliferation of hematopoietic cells. Apart from this function, FLT3LG has also shown potential as an effective antitumor agent, triggering cell survival through its synergy with several stimulating factors and interleukins, thus proving to be a critical element in the pathogenesis of a number of leukemias (eg, myeloid and lymphoid).[54]

Nosel et al[55] used microanalysis to evaluate gene expression after ex vivo IR with γ-rays ranging from 5 to 500 mGy. The number of genes that was modulated did not drastically change among the different doses, and for the lowest dose (5 mGy), they found significant decreases in the number of modulated genes with increasing post-IR times. For the higher doses (100–500 mGy), a decline in modulated genes at 5 and 7.5 hours post IR was followed by a subsequent increase. Conversely, Liu et al[56] observed in vitro dose-dependent increases in PIG3 mRNA and protein levels after IR with γ-rays ranging from 1 to 10 Gy at 8–168 hours post IR. Additionally, Paul observed a gene expression profile from ex vivo irradiated human peripheral blood samples at 6 and 24 hr post exposure. Genes significantly altered by radiation varied in biological processes including immunity, signal transduction, and apoptosis.[57]

While our study objectives were to evaluate expression changes of 24 IR-responsive candidate genes in children, we also chose to compare our results with previous IR-related studies, which are summarized in Table 2. The differences observed between our study and those previously reported can be explained by the marked difference in several experimental parameters such as radiation doses, radiation type, and studied systems (in vitro or ex vivo vs. in vivo). While we observed gene expression changes in children with very low doses from X-rays, previous studies observed changes in hematological tissue gene expression mostly through in vitro systems with variable radiation doses and γ-rays.

Previous studies detected expression changes in some genes several hours after radiation exposure and in other genes in fractions of 1 hour. Thus, multiple sampling times post-CT would have been ideal to monitor effects on RNA changes. However, this was not possible in this pilot study in particular because of the risk of jeopardizing study compliance as it would have presented a great burden on the child participants because of their young ages. Analyses post CT of 1 hour seemed the best compromise for this pilot study. However, future studies with larger sample sizes and sampling at several time intervals after CT are important for following up on our results in order to develop risk assessments, and preventive modalities for young patients undergoing CT should certainly be considered.

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Author Contributions

Conceived and designed the experiments: BMH and AAF. Analyzed the data: IP. Wrote the first draft of the manuscript: BMH and AAF. Contributed to the writing of the manuscript: MT, JFL and RVC. Agree with manuscript results and conclusions: BMH, MT, JFL, IP, RVC and AAF. Jointly developed the structure and arguments for the paper: BMH and AAF. Made critical revisions and approved final version: BMH, MT, JFL, IP, RVC and AAF.

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