CT IRRADIATION-INDUCED CHANGES OF GENE EXPRESSION WITHIN PERIPHERAL BLOOD CELLS

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Abstract—Computed tomography (CT) is a crucial element of medical imaging diagnostics. The widespread application of this technology has made CT one of the major contributors to medical radiation burden, despite the fact that doses per individual CT scan steadily decrease due to the advancement of technology. Epidemiological risk assessment of CT exposure is hampered by the fact that moderate adverse effects triggered by low doses of CT exposure are likely masked by statistical fluctuations. In light of these limitations, there is need of further insights into the biological processes induced by CT scans to complement the existing knowledge base of risk assessment. This prompted us to investigate the early transcriptomic response of ex vivo irradiated peripheral blood of three healthy individuals. Samples were irradiated employing a modern dual-source-CT-scanner with a tube voltage of 150 kV, resulting in an estimated effective dose of 9.6 mSv. RNA was isolated 1 h and 6 h after exposure, respectively, and subsequently analyzed by RNA deep sequencing. Differential gene expression analysis revealed shared upregulation of AEN, FDXR, and DDB2 6 h after exposure in all three probands. All three genes have previously been discussed as radiation responsive genes and have already been implicated in DNA damage response and cell cycle control after DNA damage. In summary, we substantiated the usefulness of AEN, FDXR, and DDB2 as RNA markers of low dose irradiation. Moreover, the upregulation of genes associated with DNA damage reminds one of the genotoxic nature of CT diagnostics even with the low doses currently applied.

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INTRODUCTION

Computed tomography (CT) was introduced to clinical routine diagnostics in the 1970s. Since then, continuous technological progress in terms of image quality and hence diagnostic power has promoted the extensive usage of CT scans, with an increase of more than 40% from 2007 to 2015 in Germany. This has led to the situation that, although the administered dose per CT examination has steadily decreased, CT scans meanwhile account for more than 60% of administered dose in the context of radiological procedures (Nekolla et al. 2017; Bundesamt für Strahlenschutz 2018). A similar tendency has also been reported for other industrial nations (OECD 2018).

This widespread application of CT has raised the need for appropriate knowledge-driven risk assessment. Yet the current base for such risk assessment is rather uncertain. In light of average doses of less than 10 mSv per CT scan, potentially increased tumor rates likely remain hidden within the statistical spread of common tumor incidence in most cases. Consequently, only a few studies have reported a possible association of radiation exposure from CT scans in childhood and adolescence and an elevated risk of cancer development in the course of a lifetime (Pearce et al. 2012; Mathews et al. 2013). The linear no-threshold (LNT) model has been introduced to circumvent the statistical limitations associated with the identification of low dose effects. Proceeding on the assumption of a linear dose-response...
relationship of ionizing radiation and carcinogenic effects, this model infers the biological impact of doses below 100 mSv from observations with higher doses. However, the validity of the LNT model has been challenged lately, especially regarding very low doses (13,14; Calabrese and O’Connor 2014; Pennington and Siegel 2019).

Against the background of these limitations and uncertainties, the spotlight has been shifted to investigations of CT effects at the cellular/molecular level. Indirect visualization of DNA double strand breaks (DSB) by means of antibodies targeting γ-H2AX, a histone modification accumulating in the course of DNA repair, revealed a linear dose-response relationship for radiation-induced damages incurred in the course of CT examinations (Lobrich et al. 2005; Rothkamm et al. 2007; Golfier et al. 2009; Kuefler et al. 2010a and b; Halm et al. 2015a; Vandevoorde et al. 2015; Popp et al. 2016; Gomolka et al. 2018). In line with the observation of CT-induced DNA DSBs, chromosomal aberrations in form of dicentric chromosomes (DIC) have also been found (Stephan et al. 2007; Golfier et al. 2009; Jost et al. 2010; M’kacher et al. 2003; Abe et al. 2015; Kanagaraj et al. 2015; Gomolka et al. 2018). However, the results of these studies are partly inconclusive with respect to the significant increase of DIC after CT scans (M’kacher et al. 2003; Stephan et al. 2007). In order to elucidate the influence of CT scans on regulatory networks and biological processes, a few studies have monitored gene expression changes in response to CT scans. Mostly, these studies followed a targeted approach based on qRT-PCR and microarray-based analysis of preselected candidate genes (Halm et al. 2015b; O’Brien et al. 2018; Port et al. 2018). To our knowledge, only one study has performed whole genome gene expression screens after in vivo CT, though with comparatively higher doses (mean radiation dose to blood of 29.8 mSv) (Nguyen et al. 2015).

In our study, starting from the controlled setting of an ex vivo exposure of peripheral blood to doses corresponding to those used in clinical routine, we characterized the transcriptomic response to CT scans by means of deep sequencing-based gene expression analysis. By analysis of three probands and two points in time post exposure, we screened for differentially expressed genes shared between independent individuals and tried to get first clues on the early dynamics of gene regulation associated with CT scans.

MATERIALS AND METHODS

Ex vivo CT irradiation and estimation of radiation dose

Peripheral blood from three healthy male donors (age at sampling: 30, 32 and 53 y) was collected into EDTA-Monovettes. Three separate blood samples for each proband and the two investigated post-exposure points in time were placed into a spine phantom set up in a 384 (2×192)-channel, Dual Source CT scanner (SOMATOM Force, Siemens Medical Solutions, Forchheim, Germany). Exposure was done according to a modified protocol for lumbar spine examinations with the following acquisition parameters: tube current 191 mAs, tube voltage 150 kV, CDTI 18, 23 mGy, and DLP 610 mGy cm⁻¹. This resulted in an administered effective dose of 9.6 mSv as estimated in accordance with the ICRP 103 protocol by means of the Monte Carlo model-based tool ImpactDose (CT Imaging GmbH, Erlangen, Germany). This tool provides scanner- and protocol-specific dose distributions based on a phantom model, which were individually adapted to the CT protocols used. After irradiation and incubation at 37 °C for 1 h and 6 h, respectively, 2.5 mL of blood were transferred to PAXgene™ Blood RNA tubes (PreAnalytiX, Hombrechtikon, Switzerland). These points in time were chosen in order to get clues on the immediate effects of CT irradiation and their dynamics. Apart from irradiation matching, non-irradiated sham control samples were handled the very same way.

RNA isolation and RNA sequencing

Total RNA was isolated using the QIAamp RNA Blood RNA Kit (PreAnalytiX, Hombrechtikon, Switzerland) on a QIAcube SP instrument (Qiagen, Hilden, Germany) following the manufacturer’s protocol. Quality and quantity of isolated total RNA were measured spectrophotometrically (NanoDrop, PeqLab Biotechnology, Erlangen, Germany). RNA integrity was assessed by the 2100 Agilent Bioanalyzer (Agilent 2100 Bioanalyzer, Agilent, Santa Clara, CA), and DNA contamination was controlled by conventional PCR using beta-actin primer. We used only RNA specimens with a ratio of A260/A280 ≥ 2.0 (NanoDrop) and RNA integrity number (RIN) ≥ 7.5 for further library preparation and sequencing.

RNA-Seq library preparation was based on the NEBNext® Ultra II Directional RNA Library Kit for Illumina following the manufacturer’s recommendations (NEBNext® Ultra II Directional RNA Library Prep Kit for Illumina and RNA depletion option; New England Biolabs Inc., Ipswich, MA; protocol version 1.0.4/17). In brief, 500 ng of total RNA was depleted of ribosomal RNA followed by cDNA synthesis and adaptor ligation (NEBNext Multiplex Oligos for Illumina; Index Primer Set 1, protocol 2016). Library quantification and assessment of length distribution was done by means of a Qubit 3.0 Fluorometer (Thermo Scientific, Waltham, MA) and an Agilent Bioanalyzer system (Agilent 2100 Bioanalyzer, Agilent, Santa Clara, CA). After successful quality control single-end sequencing (1×75 bp) was performed on the Illumina NextSeq 500 sequencing platform according to the manufacturer’s protocol (NextSeq 500 High Output Kit 1×75bp; NextSeq System Guide; NextSeq Denature and Dilute Libraries Guide; Illumina, San Diego, CA; Document # 15048776 v04, Document # 15046563 v02). During sample processing, two replicates (proband 1, second
replicate of the sham irradiated sample at the 1 h point in time; proband 3, third replicate of the irradiated sample at the 6 h point in time) have gone lost for technical reasons.

**RNA-Seq data analysis**

For differential gene expression analysis, we followed the workflow described by Love et al. (Love et al. 2014). Sequence reads were aligned to the human transcriptome, and transcript abundance was quantified (Homo_sapiens. GRCh37.67) with Salmon (version 0.8.1) (Patro et al. 2017), resulting in estimated gene counts. Count matrices were generated by tximport (version 1.4.0) (Soneson et al. 2016) and analyzed for differential gene expression by means of DESeq2 (version 1.22.2) (Love et al. 2014). Genes were assumed to be differentially expressed with log2-fold changes of FPKM >0.8 / <=−0.8 (fragments per kilobase per million mapped fragments) and P_adj (FDR-adjusted p-value) <0.1. Gene enrichment analysis was done with the web-based tool AmiGO 2 (Ashburner et al. 2000; 10,11; Mi et al. 2019) [Analysis Type: PANTHER Overrepresentation Test (Released 20190606); Annotation Version and Release Date: PANTHER version 14.1 Released 2019-03-12, Reference List Homo sapiens (all genes in database); Test Type: FISHER, False discovery rate <0.05; PANTHER Pathways, Reactome Pathways, PANTHER Protein class, PANTHER GO–Slim Molecular Function, PANTHER GO–Slim Biological Process, PANTHER GO–Cellular Component]. The data discussed in this publication have been deposited in NCBI’s Gene Expression Omnibus (Edgar et al. 2002; Barrett et al. 2013) and are accessible through GEO Series accession number GSE136310 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE136310).

**Gene expression validation using real-time polymerase chain reaction (qRT-PCR)**

For validation of RNA-Seq data by means of qRT-PCR, we selected four deregulated genes as detected by DESeq2, including upregulated genes (AEN, BARD1) as well as genes with low expression and rather poor p-values (FDXR, DDB2) (the latter two because of previous reports on their potential significance in the context of CT irradiation). In brief, aliquots of total RNA (1 μg) were reverse-transcribed with the High-Capacity cDNA Reverse Transcription Kit [Applied Biosystems (AB)]. The PCR reaction contained TaqMan Universal PCR Master Mix and one of four inventoried TaqMan minor groove binder assays (AEN, Hs00224322_m1; BARD1, Hs00184427_m1; FDXR, Hs01031617_m1; DDB2, Hs00172068_m1) for detection of candidate transcripts following the qRT-PCR 7900er amplification protocol. The qRT-PCR was performed according to the standard operating procedures implemented in our laboratory in 2008 when the Bundeswehr Institute of Radiobiology became DIN-certified (DIN EN ISO 9001/2008). All materials used were ordered from Thermo Fischer/Applied Biosystems (Weiterstadt, Germany).

**RESULTS**

**Gene expression profiles from peripheral blood lymphocytes after CT-irradiation**

The overall effect of CT irradiation on gene expression was moderate. However, the extent of response differed

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Fig. 1. Hierarchical clustering of the top 50 highest variable genes: visualization of the amount by which each gene varies in a particular sample from the gene’s average across all samples. The first and second row indicate condition and proband, respectively. The intensity of deregulation is displayed by a color gradient, with red shading implying upregulation and blue shading downregulation of log2-transformed normalized reads.

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between individuals with significantly deregulated genes per proband ranging from 5–264 at the 1-h point in time and 14–30 genes at the 6-h point in time, respectively. The search for biological pathways and processes overrepresented within the set of deregulated genes produced significant results only for the proband with the highest number of deregulated genes, where AMIGO2 revealed overrepresentations at the 1-h point in time within the set of downregulated genes for complex-I-biogenesis (p = 4.77 × 10⁻⁸), respiratory electron transport (p = 8.32 × 10⁻¹¹), oxidoreductase activity (p = 2.52 × 10⁻⁴) and transcriptional regulation in the form of RNA polymerase II regulatory region sequence-specific DNA binding (p = 3.41 × 10⁻⁴). Inter-individual differences also dominated the grouping of samples by means of unsupervised hierarchical clustering based on the 50 highest variably expressed genes (Fig. 1).

Despite these inter-individual differences, we have identified four genes with shared expression characteristics in all three probands: BARD1 (1 h), AEN (6 h), DDB2 (6 h), and FDXR (6 h). Yet it has to be stated that not all of them exceeded the commonly used thresholds of RNA-Seq analysis for fold change of >1/−1 or P_adj of >0.1 (Fig. 2). Verification experiments by means of qPCR confirmed the significant upregulation of AEN, DDB2, and FDXR in all three individuals for point in time 6-h post exposure but failed to do so for BARD1 at point in time 1 h, most likely due to the very low expression of this gene (Supplemental Digital Content Table 1, http://links.lww.com/HP/A182).

Fig. 2. Gene plots of AEN, DDB2 and FDXR at 6 h after irradiation: depicted are the RNA-Seq gene counts for each technical replicate of the three probands (G1, G2, and G3) connected by colored lines. The third replicate in proband 3 (G3) was lost during sample preparation. Fold changes (FC) and P_adj are provided in the lower left corner.

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DISCUSSION

In this study, we investigated the early transcriptomic response of peripheral blood cells taken from three healthy donors to ex vivo CT exposure. In line with the estimated low dose of 9.6 mSv, the CT associated, immediate effect on gene expression as detected by whole genome screen was expectedly moderate. Notably, despite the overall small number of deregulated genes, we have identified four genes with shared trends of CT-associated gene regulation in all three probands by RNA-Seq analysis. Technical verification by qPCR succeeded in three of them: AEN, DDB2 and FDXR. All three genes are targets of p53 (Liu and Chen 2002; Jeay et al. 2015) and have already been discussed in the context of irradiation and DNA damage response.

AEN (apoptosis enhancing nuclease), upregulated 6 h post exposure, is a radiation-induced exonuclease with activity against single- and double-stranded DNA and RNA following DNA damage. Furthermore, it mediates p53-dependent apoptosis (Lee et al. 2005; Kawase et al. 2008). AEN has been suggested as a potential biodosimetry marker as it is inducible by different doses and radiation qualities (Chauhan et al. 2014; Lu et al. 2014; Ghandhi et al. 2015; Broustas et al. 2017) and shows a correlation of gene expression with dose (Lacombe et al. 2018). This mainly applies also to the other two genes, DDB2 (damaged DNA binding protein 2) and FDXR (Ferrodoxin Reductase) (El-Saghire et al. 2013; Macaeva et al. 2018), which were also upregulated 6 h after CT exposure. Both genes were previously highlighted as irradiation responsive genes by transcriptomic studies of blood cells irradiated ex vivo and in vivo with low and high doses, respectively (Paul et al. 2011; Knops et al. 2012; Manning et al. 2013; Lucas et al. 2014; Brzóska and Kruszewski 2015; Abend et al. 2016; Port et al. 2018) and after internal irradiation (Edmondson et al. 2016). The re-identification of these genes in our analysis further substantiates their usefulness as RNA markers of irradiation.

The impact of CT exposure on these genes is less studied and understood. Halm et al. (2015b) examined gene expression changes in peripheral blood of children in-vivo irradiated with effective doses between 0.78 and 11.3 mSv 1 h after CT examination. Although DDB2 was included in their targeted gene expression analysis, no significant deregulation was detected. Similar results for DDB2 were reported by Lee et al. (2015) when examining potential gene expression changes after single photon emission computed tomography myocardial perfusion imaging (SPECT-MPI). In contrast, significant deregulation of DDB2 could be demonstrated after CT angiography examinations. One explanation for this difference might be the rather high median effective dose of 36.9 mSv applied in these angiography examinations (Nguyen et al. 2015). As to FDXR, O’Brien and colleagues demonstrated upregulation as early as 2 h after irradiation with effective doses ranging from 8 mSv up to 21 mSv (O’Brien et al. 2018). Finally, Port et al. proved up-regulation of FDXR and DDB2 24 h after irradiation with effective CT doses between 4–18 mSv (mean dose of 9.5 mSv) (Port et al. 2018), which matches our results achieved with comparable dose settings.

Unfortunately, we have failed to verify RNA-Seq data on the upregulation of BARD1 1 h after exposure. The most likely explanation for this is the extremely low expression of this gene challenging the technical limits of our whole genome expression screen. Nevertheless, we think that this gene merits further investigation in the context of low dose irradiation since BARD1, in complex with BRCA1, is a versatile ubiquitin ligase implicated in a broad spectrum of biological processes ranging from DNA damage response (Lee et al. 2015a; Zhao et al. 2017; Adamovich et al. 2019; Nakamura et al. 2019) to cell cycle control after DNA damage (Fabbro et al. 2004).

CONCLUSION

Albeit the impact on early gene expression signatures was rather low and dominated by inter-individual differences, we were able to extract three genes with common response to CT exposure. All of them have been implicated previously in DNA damage response and DNA repair. This indicates that CT, even with the low doses applied currently, remains a genotoxic stressor. The re-identification of three genes previously implicated in irradiation response underscores their usefulness as robust RNA markers for low-dose irradiation.

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