Effect of ionizing radiation on cellular procoagulability and co-ordinated gene alterations

Petra Goldin-Lang, Klaus Pels, Quoc-Viet Tran, Bjoern Szotowski, Frank Wittchen, Silvio Antoniak, Tobias Willich, Henning Witt, Michael Hummel, Dido Lenze, Wolfgang Poller, Heinz-Peter Schultheiss, Ursula Rauch

From the Department of Cardiology and Pneumology, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany (PG-L, KP, Q-VT, BS, FW, SA, TW, WP, H-PS, UR); Center for Cardiovascular Research, Charité-Universitätsmedizin Berlin, Campus Mitte, Berlin, Germany (HW); Department of Pathology, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany (MH, DL).

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Correspondence:
Ursula Rauch, MD, Medical Clinic II, Charité-Universitätsmedizin Berlin Campus Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany.
E-mail: ursula.rauch@charite.de

ABSTRACT

Background and Objectives

Ionizing radiation (IR) is associated with thrombotic vascular occlusion predicting a poor clinical outcome. Our study examined whether IR induced tissue factor (TF) expression and procoagulability. We further investigated coordinated gene alterations associated with TF upregulation in the myelomonocytic leukemia THP-1 cells.

Design and Methods

TF expression was determined by quantitative Reverse Transcriptase (TaqMan®) PCR, TF ELISA and TF activity by a two stage chromogenic assay in the time course of days 1, 3, 7, 10, and 17 post IR. To detect IR-induced alterations in gene expression, Affymetrix HG U133 Plus 2.0 microarrays were used.

Results

IR induced a significant increase in TF/GAPDH mRNA ratios and cellular TF protein on days 3 and 7 post IR (20 Gy [$p \leq 0.01$] and 40 Gy [$p \leq 0.01$ vs. control]), suggesting a late and persistent induction of TF. An increase in cellular TF activity was already found 1 day post IR (20 Gy and 40 Gy [$p \geq 0.001$] vs. control respectively), suggesting IR immediately alters the cellular thrombogenicity. TF upregulation post IR was confirmed in PBMNCs. Gene expression profiling showed IR increased the expression of inflammatory and apoptosis-related pathways known to be involved in the regulation of TF expression.

Interpretation and Conclusions

TF upregulation together with inflammation and apoptosis may increase the thrombogenicity of tissues. The demonstrated upregulation of TF might play a pivotal role in radiation associated thrombosis..

Key words: gene expression profiles, ionizing radiation, myelomonocytic cell line THP-1, procoagulability, tissue factor.

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Ionizing radiation (IR) is associated with an increased risk of thrombotic occlusion of vessels and organ fibrosis.[1] Thrombotic events have been described as a major complication after IR.[2] Cells surviving acute genotoxic stress post IR have been shown to display delayed responses that can result in persistent effects such as apoptosis and late thrombosis.[3,4] Overexpression and increased activity[5] of tissue factor (TF) have been shown to be involved in radiation-induced changes.[6] Furthermore, cultures of human arterial endothelial cells expressed TF mRNA after irradiation in combination with mechanical denudation.[7]

Alterations in transcriptional factor activity may contribute to the increased thrombogenicity present post IR.1 Sreekumar *et al.*[8] found TF protein to be upregulated in response to radiation treatment as shown by application of microarrays in LoVo colon carcinoma cells. TF, the initiator of the extrinsic coagulation system, induces thrombus formation by activation of Factor VII resulting in activation of Factors IX and X. TF is widely thought to play a leading role in thrombus formation during thrombotic disorders.[9–13] Besides its role in hemostasis,[13,14] upregulation of TF expression appears to be characteristic of tumor tissue. There, TF is expressed in malignant cells as well as in tumor-infiltrating macrophages and endothelial cells.[15,16,17] The procoagulant activity of TF appears to play an important role in the development of disseminated intravascular disease (DIC).[18] It has been shown that platelet activation and fibrin deposition is an essential part of TF-dependent metastasis.[19] In addition, blood clotting abnormalities are detected in up to 90% of patients with metastatic disease, and thrombosis represents the most frequent cause of cancer mortality.[20,21]

Pathologic activation of the coagulation cascades by aberrant expression of TF on the surface of monocytes has been implicated in life-threatening thrombosis.[22] TF is expressed on the surface of human leukocytes and leukemic myelomonocytic cells[23,24] where it has been reported to increase cellular thrombogenicity. In this study, THP-1 cells were used as a model because of their cytologic, histochemical, and functional properties which resemble human monocytes.

In response to IR, monocytes have been reported to produce inflammatory cytokines such as TNF-α. NFκB, one of the main mediators of cellular responses involved in inflammation, apoptosis and regulation of TF expression, was documented to be activated by IR through a cascade requiring endogenous TNF-α production.[25]

The aim of our study was to clarify whether IR induces the expression of procoagulant proteins such as TF in the THP-1 model over a time period of 17 days. In a second step, we examined coordinated gene alterations associated with IR-induced thrombogenicity and increased TF procoagulability on day 7 post IR.

Design and Methods

Human myelomonocytic leukemia cell line

The human monocytic cell line THP-1 has been purchased from LGC Promochem (Wesel, Germany). Cells were cultured in RPMI 1640 (GIBCO, Karlsruhe, Germany) supplemented with 10% Fetal Calf Serum (FCS) (GIBCO, Karlsruhe, Germany), 1mM L-glutamine (GIBCO, Karlsruhe, Germany), and 1% penicillin/streptomycin (GIBCO, Karlsruhe, Germany). They were tested and found to be mycoplasma and endotoxin free. Cell viability was assessed by trypan blue dye exclusion prior to all treatments.

Isolation of human peripheral blood mononuclear cells (PBMNCs) and human PBMNC culture

Human mononuclear cells were separated from blood samples as follows: 18 mL of EDTA treated human peripheral blood was diluted (1:2) in warm (37°C) phosphate-buffered saline (PBS, without Ca^{2+} and Mg^{2+}) and purified by the Ficoll-Paque Plus. Then 7 mL of Ficoll-Paque were overlayed with 7 mL of the PBS-blood mixture and centrifuged (610×g, 25 mins., room temperature, without brake). The cell layer over the Ficoll-Paque was collected and washed two times in 10 mL PBS and centrifuged (610×g, 10 mins., room temperature). After the last wash, the cell pellet was resuspended in 500 µL RPMI 1640 supplemented with 10% Fetal Calf Serum (FCS), 1mM L-glutamine, and 1% penicillin/streptomycin. It was tested and found to be mycoplasma and endotoxin free. Cell viability was assessed by trypan blue dye exclusion prior to all treatments. For downstream applications, cells were counted in a hematocytometer (MICROS®60-OT System, Axonlab, Stuttgart).

Irradiation of THP-1 cells

THP-1 cells (10^6/mL) were irradiated with a single IR dose of either 20 or 40 Gray. IR was generated by a linear accelerator (Varian Clinac 600 CD) with a maximum photon energy of 6 MeV. Cell cultures were irradiated in a water-equivalent-environment by a 25×25 cm^3 photon field. With this set-up, the dose homogeneity in the cell culture media is in a range of 100-105%. Following irradiation, cells were maintained in growth medium and afterwards subjected to further analysis. To evaluate the effect of the NFκB-pathway, inhibition experiments with BAY 11-7082 were performed with 4×10^5 THP-1 cells/mL. These were incubated 1h pre IR and daily up to 3 days post IR with 5 µM BAY 11-7082. Furthermore, THP-1 cells pre and post IR were subjected to RNA extraction and QRTPCR analysis.

Tissue factor ELISA

To quantify the total TF protein content in irradiated and control cells, THP-1 cells were subjected to ELISA according to the manufacturer's instructions (Imubind Tissue Factor ELISA Kit, American Diagnostica, Pfungstadt, Germany).

Two stage chromogenic tissue factor activity assay

Measurement of TF activity was performed as previously described.[14] Briefly, THP-1 cells were washed twice with phosphate buffered saline (PBS), incubated in HEPES buffer containing 0.1 M n-octyl-β-D-glycopyranosid. After addition of Factor VIIa, Factor X and $Ca2^+$ TF-dependent Factor Xa generation was measured at 405 nm using a chromogenic substrate for Factor Xa. TF activity units were assessed by a standard curve. The standard curve is constructed by plotting the mean slope absorbance value measured for each lipidated TF standard against its corresponding concentration [pg/mL] according to the manufacturer's Actichrome® TF instruction sheet (American Diagnostica, Pfungstadt, Germany). The activity (generation of Factor Xa) exhibited by 1 pg of lipidated TF corresponds to 1 arbitrary TF-activity unit.

RNA extraction, reverse transcription

Total RNA of THP-1 cells or PBMNCs (10^6) was isolated by RNeasy Mini Kit including Qiashredder columns (Qiagen, Hilden, Germany). DNase I (Fermentas, St. Leon-Rot, Germany) digested, reverse transcription of RNA was performed with a First strand cDNA synthesis kit for RT-PCR (AMV; Roche Applied Sciences) according to manufacturer's instructions.

Quantitative real-time PCR (QRTPCR)

PCR conditions for the TaqMan® (ABI Prism7000 Sequence Detection System, Applied Biosystems, Darmstadt, Germany) are summarized in Table 1. Primers and probes were purchased from TIB Molbiol (Berlin, Germany). Data were analyzed using SDS 7000 software.

GeneChip oligonucleotide microarrays target preparation

GeneChips (HG U133 Plus 2.0) were purchased from Affymetrix (Santa Clara, CA, USA). Target preparation and microarray processing were carried out according to the manufacturer's recommendations. Briefly, 3 μg of total RNA was used to prepare double-stranded cDNA. Biotinylated cRNA was synthesized with an RNA transcript labeling kit and 20 μg of the cRNA product was chemically fragmented to approximately 50-200 nucleotides. Quality of RNA was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies GmbH, Waldbronn, Germany). The target for hybridization was prepared by combining 20 μg of fragmented cRNA and was hybridized to an Affymetrix HG U133 Plus 2.0 chip. Chips were washed and stained using the EukGE-WS2v5 protocol on an Affymetrix fluidics 450 station. The stain included streptavidin-phycoerythrin (10 μg/mL) and biotinylated goat anti-streptavidin (3 μg/mL). Fluorescence intensities were captured with an argonion laser confocal scanner.

Analysis of GeneChip data

Scanned output files were analyzed with GeneChip Operating Software 1.2 (GCOS; Affymetrix, Santa Clara, CA, USA). Fluorescence intensity was measured for each chip and normalized to the average fluorescence intensity for the entire chip. The normalized data set from three independent replicates (day 7 post IR; untreated controls versus irradiated THP-1 cells with a 20 Gy dose) was used for the Significance Analysis of Microarrays (SAM).[26] The SAM statistic identifies significant changes in gene expression by performing a set of gene-specific t-tests. A score is calculated for each gene on the basis of changes in its expression relative to the standard of repeated measurements for that gene. Genes with scores greater than a threshold Δ are defined as significantly deregulated. A false discovery rate can be estimated from random permutations of all measurements. A cut-off of 1.8-fold expression (q-value ≤5%, only for TF gene: q-value >5%) was set to iden-

Table 1A. Primer and Probe Sequences, conditions and performance (50°C, 2min; 95°C, 10min; 40 cycles 95°C, 15s, 60°C, 1min) of quantitative real-time RT- PCR (QRTPCR) and conventional RT- PCR (cDNA standards for real time RT-PCR).

	Primer and probe (100 nm) sequences	*Amplicons (bp)*
HTF upper (sea):	5'- GAA GCA GAC GTA CTT GGC ACG G	
HTF lower (asb):	5'- CCG AGG TTT GTC TCC AGG TA	126
HTF So2:	5'- FAM-CAG GGA ATG TGG AGA GCA CCG GTT CTG T X[c]T--PH	
HGAPDH T fwd (se[a]):	5'- CCA CCC ATG GCA AAT TCC	
HGAPDH T rev 2(as[b]):	5'- TCG CTC CTG GAA GAT GGT G	250
HGAPDH So:	5'- FAM-TGG CAC CGT CAA GGC TGA GAA CGT X[c]T--PH	

Primer sequences, conditions and performance of conventional RT- PCR (cDNA standards for real time RT-PCR).

human tissue factor (TF):
initial denaturation: 94°C, 2 min, 94°C, 30 s, 60°C, 30s ; 72°C, 90 s for 30 cycles; final extension: 72°C, 5 min
human GAPDH:
initial denaturation: 94°C, 2 min, 94°C, 30 s, 58°C, 30s ; 72°C, 90 s for 30 cycles; final extension: 72°C, 5 min

HTF left1:	5'-CGC CGC CAA CTGGTAGAC	931
HTFright1:	5'-TGC AGT AGC TCC AAC AGT GC	
HGAPDH stFOR:	5'-GCC ACA TCG CTC AGA CAC CAT	1229
HGAPDH stREV:	5'-GGT TGA GCA CAG GGT ACT TTA TTG ATG	

[a]se: sense; [b]as: antisense; cX:TAMRA.

Table 1B. Primers for QRTPCR analysis of expression levels of selected genes of IR exposed THP-1 cells.

Gene	*Forward primer*	*Reverse primer*
TNFRSF10B	GGCCAAATAGCATGTGACACA	AGGGCAGAAGCATGAAAGGA
P21/CIP1	CAGCGACCTTCCTCATCCA	CCTTGTTCCGCTGCTAATCAA
TNFSF13B	ATGCCTGAAACACTACCCAATAATT	GCAAGTTGGAGTTCATCTCCTTCT
IFI27	TGTCATTGCGAGGTTCTACTAGCT	CCCCTGGCATGGTTCTCTT
CTSD	GAGCCGTGGAGGATGCAA	GGAAGATGAAGACGAGGATGATG
PLA2G4C	CACTGTGGGCCTGTTGGTCTA	CTGAAGGGAGTGAAGAACAGGAA
HGAPDH	CCA CCC ATG GCA AAT TCC	TCG CTC CTG GAA GAT GGT G

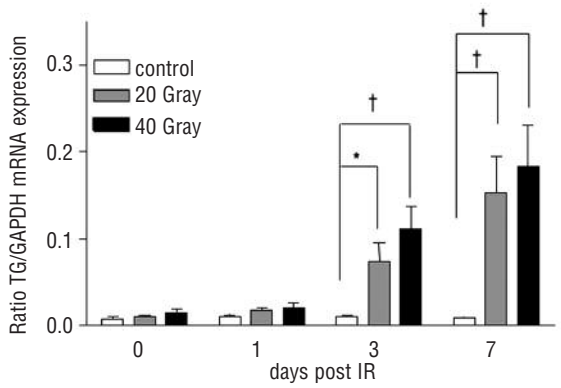


Figure 1. TF mRNA expression in THP-1 cells pre and 1, 3, 7 days post ionizing radiation (IR) with 0, 20 and 40 Gy. Upregulated TF mRNA 3 days post IR with doses of 20 and 40 Gy *$p \leq 0.05$ †$p \leq 0.01$ vs. control; ‡$p \leq 0.001$ vs. control.

tify genes whose expression was significantly differentially regulated.

Statistical analysis

Data analysis was performed using SPSS 12.0. The Kolmogorov-Smirnov-Test was performed to test data distribution. Values are presented as mean ± standard error of mean (SEM) or median and interquartile range for non-parametric data. For parametric data, statistical significance of differences between groups was determined by applying the unpaired Student *t*-test or one-way analysis of variance (ANOVA) for multiple comparisons. The Mann-Whitney U-test was performed for non-parametric data: $p<0.05$ was considered significant. All experiments were performed at least five times.

Results

IR-induced upregulation of TF mRNA, protein and procoagulability in human monocytic cells

QRTPCR was performed on irradiated and non-irradiated THP-1 cells to investigate the effect of IR on TF expression. IR led to an increased mRNA expression of TF in irradiated THP-1 cells (Figure 1). On day 3 post IR (control vs. 20 Gy [$p \leq 0.05$] and vs. 40 Gy [$p \leq 0.01$]) and on day 7 post IR, *TF/GAPDH* mRNA ratios gave the highest mRNA expression levels (control vs. 20 Gy [$p \leq 0.01$] and vs. 40 Gy [$p \leq 0.01$], repectively) indicating a late induction of TF. PBMNCs were isolated from human peripheral blood to analyze the TF mRNA expression of mononuclear cells compared with the THP-1 cell model. A significant increase in TF/GAPDH mRNA ratios was found 1 day post IR (0.00041±0.00006 vs. 0.0022±0.0006, baseline vs. 20 Gy, $p=0.008$) and 3 days post IR of PBMNCs (0.00041±0.00006 vs. 0.006±0.002, baseline vs. 20 Gy, $p=0.0016$).

To analyze the NFκB-pathway related TF expression post IR, we performed mRNA expression studies by using the NFκB inhibitor BAY 11-7082. This inhibits IκBα phosphorylation. An inhibitor concentration of 5 μM had almost no effect on the growth of THP-1 cells. A significant inhibition of TF mRNA expression in irradiated cells pretreated with BAY 11-7082 was seen 3 days post IR compared to irradiated cells cultured without NFκB inhibitor (ratio HTF/HGAPDH 0.010±0.001 vs. 0.066±0.018; $p \leq 0.0001$). At day 3, when THP-1 cells were pretreated with NFκB inhibitor without application of IR, the TF mRNA expression of the NFκB inhibitor-pretreated cells was significantly downregulated compared to untreated control cells (ratio HTF/HGAPDH 0.009±0.001 vs. 0.012±0.001; $p \geq 0.05$). However, the application of IR without pretreatment with BAY11-7082 was associated with an increased TF mRNA expression compared to non-irradiated and untreated control cells 3 days post IR (ratio HTF/HGAPDH 0.066±0.018 vs. 0.012±0.001; $p \leq 0.001$). To confirm the data obtained on mRNA level, IR-induced expression of TF protein in THP-1 cells was quantified by ELISA recognizing human TF. A significant increase in cellular TF protein became prominent 3 days post IR with 20 Gy and with 40 Gy and persisted throughout the period of study (Figure 2A). A significant increase in cellular TF activity was already found 1 day post IR with 20 Gy and with 40 Gy, indicating IR alters cellular thrombogenicity (Figure 2B). A 12-fold increase was seen 7 days post IR with a dose of 20 Gy (Figure 2B, $p \leq 0.001$) and 40 Gy ($p \leq 0.001$) compared with untreated THP-1 control cells. Increased cellular prothrombogenicity was persistently measurable throughout the period of study (day 0-day 17) (Figure 2B).

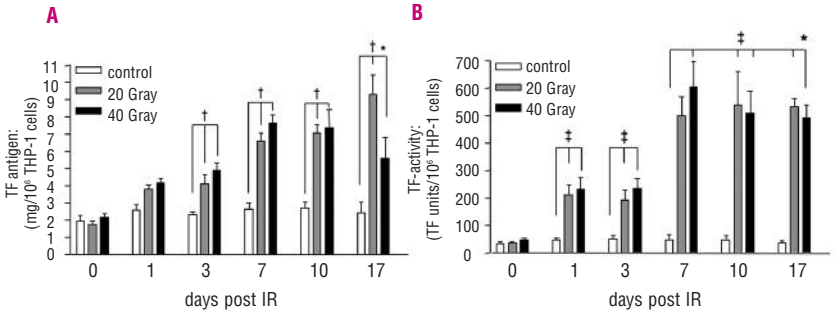
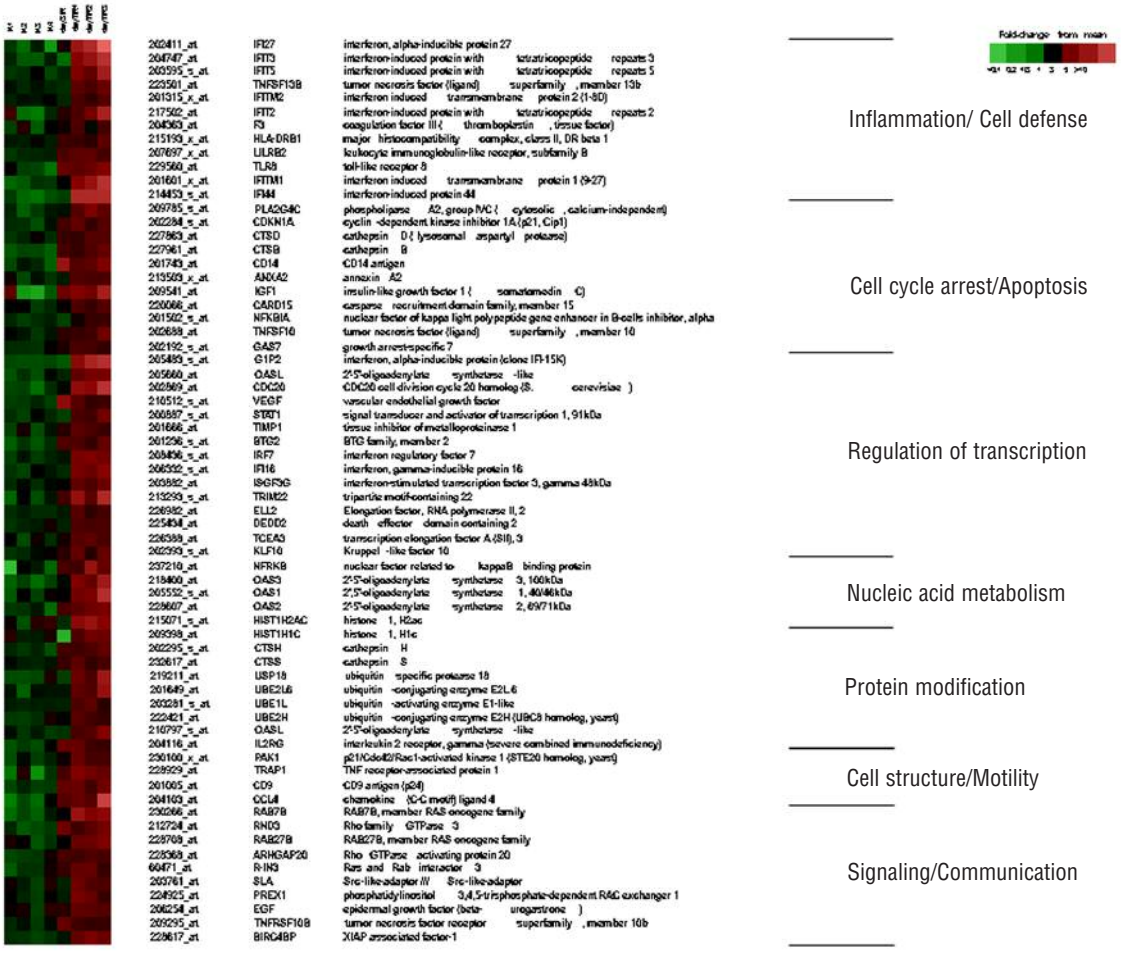


Figure 2. A. TF protein expression pre and 1, 3, 7, 10 and 17 days post ionizing radiation (IR) with 0, 20 and 40 Gy. Ionizing radiation-induced TF protein synthesis of THP-1 monocytic cells 3 days post IR. *$p \leq 0.05$; †$p \leq 0.01$ vs. control. **B.** TF associated procoagulant activity pre and 1, 3, 7, 10 and 17 days post IR. TF procoagulant activity increased 1 day post IR with doses of 0, 20 and 40 Gy. *$p \leq 0.05$; †$p \leq 0.01$; ‡$p \leq 0.001$ vs. control.

Table 2. Differentially expressed genes in irradiated THP-1 cells. Data from individual samples for genes arranged in functional groupings are shown. K1-4: non-irradiated controls (replicates 1-4); day3IR, day7IR1-3: irradiated THP-1 cells (20 Gy); day3,7 post IR.



Microarray analysis post IR: altered transcriptional Tissue Factor and gene expression profiles

Genechip microarray analysis was performed to clarify possible mechanisms underlying the increased expression of TF in THP-1 cells post IR.

The data discussed in this publication have been deposited in NCBIs Gene Expression Omnibus (GEO, *http://www.ncbi.nlm.nih.gov/geo/*) and are accessible through GEO Series access number GSE4110. We found a panel of candidate genes to be upregulated on day 3 and day 7 post IR. We performed only one pair of microarray analysis with day 3 post IR and the untreated control. On day 3 post IR, a generally weaker expression pattern of differentially expressed genes in irradiated THP-1 cells was detected. Therefore, 3 further replicate pairs (treated vs. untreated state) were chosen for the SAM statistics (Table 2). The number of genes with elevated expression increased in relation to an increased duration of culture time post IR. All detected 422 gene transcripts with known functions and significant upregulation (>1.8-fold, q≤5%) on day 7 post IR compared to non-irradiated controls are available in the online supplement. Altered transcription patterns post IR revealed genes belonging to the following groups: inflammation/cell defense, cell cycle arrest/apoptosis, regulation of transcription, nucleic acid metabolism, protein modification, cell structure/motility, and signaling/communication (Table 2).

IR-responsive gene expression involved in regulation of cell thrombogenicity

We focussed on genes involved in inflammatory and apoptotic pathways which are known to be linked to changes in cellular thrombogenicity (Table 3). To validate upregulation of these candidate transcripts, SYBR Green QRTPCR was performed as well as the microarrays (Table 3).

The group IV phospholipase A2 (group IVC, cytosolic, Ca^{2+}-independent; PLA2G4C) gene encodes for an enzyme important in membrane remodelling, activation and symmetry of lipid membrane, which is involved in cell thrombogenicity. QRTPCR confirmed a 4.1-fold

Table 3. Comparison of relative expression levels measured by microarray analysis and by SYBR Green QRTPCR for selected genes.

		Fold change micro-array		*Fold change control/ 7 days post IR (20 Gy)*		*Putative effect on*	
Gene ID	*Gene name*	*day 3*	*day 7*	*QRT-PCR*	*q-value [%]*	*Signaling*	*Apoptosis*
209785_s_at	phospholipase A2 (group IVC, cytosolic, Ca-independent; PLA2G4C	–	3.6	4.1	0.62	–	+
202284_s_at	P21/CIP1	2.6	2.6	6.4	1.59	–	(-) cell proliferation
227863_at	Cathepsin D; CTSD	–	2.2	3.9	2.85	–	+
209295_¬at (TRAIL R2/Killer/DR5)	TNFRSF10B 2.3	1.8	6.3	4.0	–	+	
223502_s_at	TNFSF13B	–	3.5	17.1	0	+	–
202411_at	IFN-α inducible protein 27; IFI27	2.3	20	166	0	+	–

upregulation of the group IV phospholipase A2 gene. QRTPCR also confirmed upregulation of cathepsin D (CTSD), an inducer of apoptosis in monocytes (Table 3).

Since TF is upregulated in monocytes under inflammatory conditions, especially by stimulation with TNF-α, we further verified the upregulation of TNFRSF10B (TRAIL R2/APO2LR/Killer/DR5). This is a gene for TNF-related apoptosis-inducing ligand receptor 2 belonging to the cell signaling/communication group, known to be caspase-dependent and associated with apoptotic pathways (Table 3). QRTPCR showed IR clearly increased production of TNF (ligand) superfamily, member 13B (TNFSF13B) gene, a TNF-and APOL-related leukocyte expressed ligand 1, which is a TNF homolog activating the NFκB-, the JNK pathway and inducing apoptosis (Table 3). IFN-α inducible protein 27 (IFI27) gene was also seen to be highly upregulated post IR (Table 3).

We found three genes (*TNFRSF10B, TNFSF13B* and *IFN-α inducible protein 27*) whose regulation according to QRTPCR was considerably greater. This was also true of the *TF* gene, which was 1.8-fold (q-value > 5%) upregulated in the microarray experiment compared to a 3-fold upregulation measured by SYBR Green QRTPCR.

Discussion

In the present study IR was found to induce a persistent upregulation of TF on mRNA and protein level as well as TF procoagulant activity. Gene expression profiling showed IR also upregulated inflammatory and apoptosis-related pathways. TF activity was already upregulated on day 1 post IR, while increased TF protein and mRNA expression were found on day 3 in PBMNCs and in THP-1 cells. Although in many tumor cells all functional TF molecules are localized on the outer cell membrane, the procoagulant activity on the intact cell surface is largely dormant and can be enhanced by cell injury or damage.[27] Bach *et al*.[28] described cellular TF activity to be *encrypted*. TF may be deencrypted in several ways, for example by non-ionic detergents or calcium ionophores, thus leading to an increased TF activity without increasing TF mRNA or antigen expression. IR-induced activation of constitutively expressed TF on THP-1 cells may account for the early increase in procoagulant activity which was measurable before upregulated TF mRNA levels were found. As far as the extent of TF upregulation is concerned, a correlation was seen between the fold changes in normalized intensities as determined by microarrays and by QRTPCR analysis (Table 3). However, the magnitude of gene upregulation measured by SYBR Green QRTPCR was considerably higher in some of the candidate genes (Table 3). Compared to QRTPCR, the probeset for the TF gene on the microarray was not as sufficient for hybridization with cRNA of monocytic THP-1 cells potentially leading to weak signal intensities and lower ratio. Expression levels detected by QRTPCR were generally stronger than those obtained by the cDNA microarray. This fact may explain the observed differences in gene expression levels as presented here (Table 3). To widen out knowledge of the mechanisms and pathways involved in the delayed response to irradiation, gene expression patterns in irradiated and non-irradiated THP-1 cells were compared. We found an upregulation of a phospholipase A2 (*PLA2*) gene. This is involved in the assembly of membranes with coordinate synthesis, catabolism, and transport of phospholipids. In whole blood, activation of transcription factors is mediated through the phospholipase A2 pathway. At the surface of viable cells, the transmembrane phospholipid dis-

tribution and its regulation may be important for the expression of the catalytic activity of the complex of TF and activated Factor VII.[29] Furthermore, phosphatidylserine supports the activity of the cell surface-complex of TF/FVIIa. Recent studies have shown that phosphatidylserine exposure is one of the earliest manifestations of apoptosis, and that it precedes loss of membrane integrity.[30] Other cell death markers such as cathepsin D (CTSD) were also found to be increased post IR.

Several transcripts for interferon-inducible proteins along with those for *IFN-α* (*IFN-α* inducible protein 27; *IFI27*) and *IFN-γ* showed elevated expression levels on day 3 and on day 7 post IR. *IFN-α* was reported to increase TNF-α induced apoptosis and, therefore, monocytes have been shown to rapidly undergo apoptosis in cultures. By contrast, endogenously produced *TNF-α* after γ-irradiation was reported to result in enhanced monocyte survival by reducing induction of apoptosis.[31] Many downstream targets of interferon were gradually induced following IR treatment. It is known that interferon operates through the JAK-STAT pathway in response to viral infections to mediate transcriptional changes in target genes. This results in antiproliferative effects, involved in suppressing viral replication. In case of IR stress, interferon activity may promote the same effects to prevent propagation of DNA damage.[32] *TNFRSF10B* (*TRAILR2*) was found to be upregulated post IR. This TNF-α receptor is a potential activator of the NFκB pathway.[33] It is known that increased expression of the TF gene resulting in increased procoagulability is regulated via NFκB by various transcription factors, including NFκB/Rel proteins and Egr-1.[34] Hachiya and co-workers[25] ahowed that irradiation increased the NFκB binding activity and increased the production of TNF-α in THP-1 cells. Endogenous production of TNF-α is known to be required for NFκB activation post IR.[35] Treatment of these cells with anti-TNF-α antibodies blocked the activation of NFκB induced by irradiation and exogenously added TNF-α stimulated NFκB activation. This is in line with the inhibition of TF mRNA expression in THP-1 cells using the NFκB pathway inhibitor BAY 11-7082 as shown here.

In conclusion, irradiation of human monocytic cells induced the upregulation of TF expression and procoagulant activity. The findings on upregulated TF mRNA expression in PBMNCs post IR are in line with our data obtained from irradiated THP-1 cells. We conclude that the THP-1 cells are a suitable model for studying the effect of various agonists on TF expression levels in human mononuclear cells. The demonstrated upregulation of TF and other regulatory pathways might play a pivotal role in radiation associated thrombosis and further studies are warranted to investigate this hypothesis.

Authors' Contributions

PG-L: writing the manuscript, interpretation of data, radiation experiments, TF TaqMan® PCR, microarray experiments; KP: contributed equally to the 1 st author's work; Q-VT: radiation experiments, TF ELISA, TF activity assay; BS: critical revision of the manuscript and interpretation of data, discussion; FW: SAM statistics of microarray experiments; SA: validation experiments (SYBR Green TaqMan® PCR); TW: statistical analyses of TF mRNA-, TF ELISA-, and TF activity data; HW: critical revising microarray data and SAM statistics, online submission of microarray data into GEO; MH: providing of the Affymetrix working station, support in microarray experiments; DL: providing of the Affymetrix working station, support in microarray experiments; WP: critical revision of the manuscript, discussion, partly writing the manuscript, final approval of the manuscript; H-PS: critical revision of the manuscript, partly writing the manuscript, final approval of the manuscript; UR: main study concept, methods and design, critical revision and interpretation of data, partly writing the manuscript, final approval of the manuscript.

Conflict of Interest

The authors reported no potential conflicts of interest.

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