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D9.98 - Identification of human EV-related leukaemia markers and radiation exposure markers

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Abstract

This deliverable aims at investigating markers of radiation exposure in humans as well as discovering markers of leukaemogenesis by analysing the cargo of extracellular vesicles (EVs) from peripheral blood. Concerning protein content, six downregulated and 21 upregulated proteins were identified *via* high performance liquid chromatography coupled to mass spectrometry (LC-MS^n) when the serum-derived EVs of leukaemic patients were compared against healthy controls. The deregulated proteins clustered around serum amyloids, alpha-acid glycoproteins, and plasma proteases. Similarly, 14 proteins were found downregulated and 23 proteins were found upregulated in the EVs of patients after they were exposed to 2 × 2 Gy ionising radiation (IR). The deregulated proteins could be possible biomarkers of leukaemogenesis and of radiation exposure in humans.

Content

Introduction

Extracellular vesicles (EVs) are cell-derived membrane-bound vesicles in the range of 100-1000 nm that contain a complex cargo, including but not limited to lipids, nucleic acids, and protein (for example TSG-101, or membrane proteins like Tetraspanins, e.g. CD9, CD81). EVs also play a role in cell-cell communication and bystander signalling, a phenomenon where non-irradiated cells show radiation-induced damage due to irradiation of other (neighbouring) cells. They can transfer these information´s by travelling in the extracellular space and blood system to other cells. The release of EVs is significantly increased in the microenvironment of cancer[1] or under stress conditions, also with irradiation[2]. Furthermore, irradiation can influence the composition of the EVs[3].
In the LEU-TRACK project, the proteome alterations in human serum-derived EVs are being studied to discover leukaemogenesis-related biomarkers in human subjects. In addition, proteins that may serve as biomarkers for radiation exposure are also being investigated. Thus, the present deliverable aims to provide data on differences in the serum-derived EV proteomes between healthy controls and leukemic patients (EV biomarkers of leukaemia), differences in the serum-derived EV proteomes after exposure to total body irradiation (EV biomarkers of radiation) as well as differences in EV miRNA content between healthy controls and leukemic patients being irradiated.

1. Isolation and characterization of EVs from leukaemic patients and healthy donors

1.1 Method

EVs were isolated from either serum samples of healthy donors and leukaemic patients by ultracentrifugation. The leukaemic patients, stationary at the Bone Marrow Transplantation Unit, Department of Internal Medicine I, University Clinics, Goethe University were treated with 4 x 2 Gy total body irradiation (TBI) using a linear accelerator (Elekta Synergy, Elekta, Crawley, UK) for conditioning to allogenic stem cell transplantation. Samples (7.5 ml EDTA blood and 2 × 7.5 ml serum) of the leukaemic patients were collected before irradiation (d0) and after irradiation with 2 x 2 Gy (d1) (Figure 1) while blood of healthy volunteers was collected in parallel.

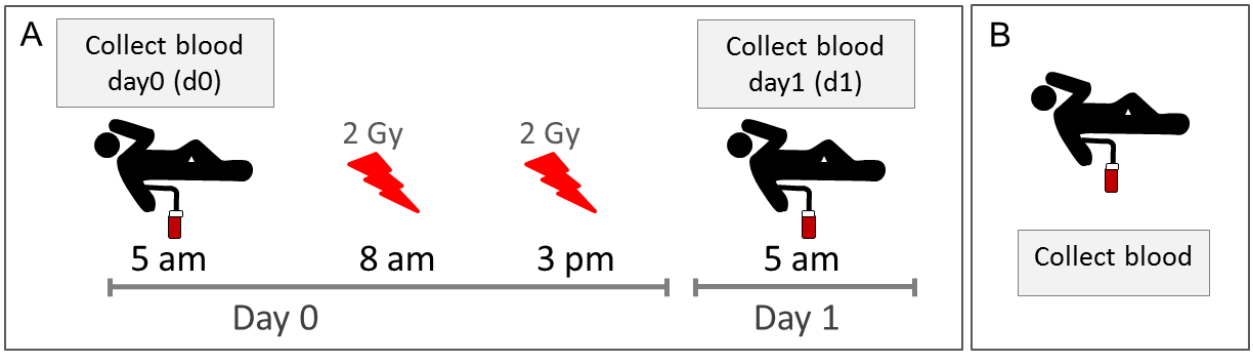

Figure 1. *Blood collection of leukaemic patients (A) and healthy donors (B).*

Various kits (ExoQuick, Exo Quick ULTRA, System Biosciences) were tested to recover the EVs from human serum. The isolated EVs, however, were contaminated with polymers and thus not suitable for proteomic analysis. For this reason, an ultracentrifugation (Optima-XPN 80, Beckmann Coulter) protocol was established. First, serum was collected and centrifuged at 1.000 x g for 15 min at room temperature (RT). Next the supernatant was centrifuged at 3.000 x g for 15 min, followed by a repeated centrifugation at 10.000 x g for 30 min to remove all cellular particles. Finally, the EVs were collected by centrifugation at 100.000 x g for 2 h at 4 °C and EVs were resuspended in 100 µl Phosphate-Buffered Saline (PBS) (Figure 2).

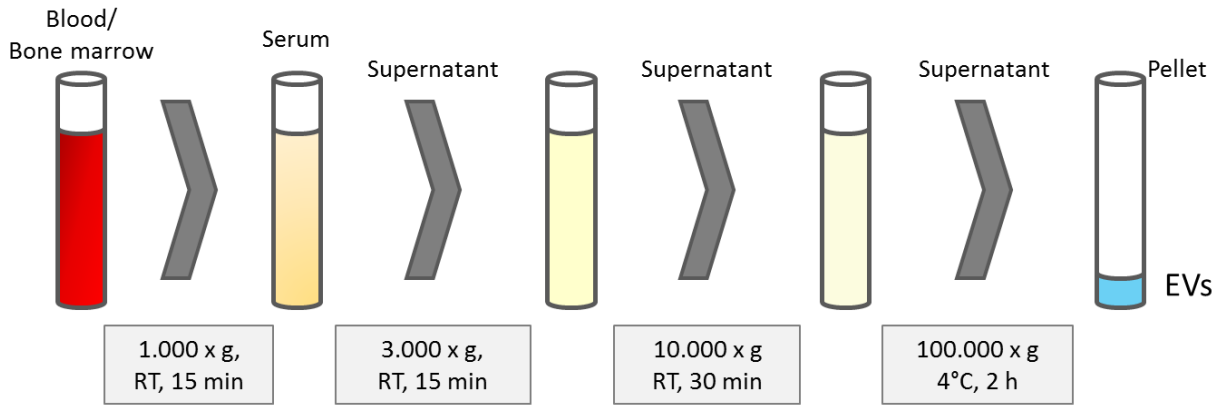

Figure 2. *Protocol of EV isolation by ultracentrifugation.*

1.2 Characterization of the EVs by Western immunoblot and Flow cytometry

The EVs from leukaemic patients and healthy donors were characterized by western blot analyses. Equal amounts of protein (50 µg) as measured by a Micro BCA™ Protein Assay Kit (Thermo Fisher Scientific, Frankfurt, Germany) were loaded onto a SDS-PAGE gel, along with molecular weight marker ProSieve QuadColor Protein Marker (Biozym Scientific). Proteins were transferred to a nitrocellulose membrane by Semi-Dry Transfer Unit (Thermo Fischer Scientific) and membranes were blocked for 1 h at RT at 5 % skim milk in Tris-buffered saline with Tween20. After washing, the membrane was stained with corresponding first antibody for 16 h at 4 °C (Table 1), incubated with secondary antibody (goat anti-rabbit IgG HRP, Southern Biotech # 4050-05 (1:1000) or goat anti-mouse IgG HRP, Southern Biotech #4050-05 (1:1000)) for 1 h at RT and developed by Pierce ECL Western Blotting Substrate (Thermo Fischer Scientific) or WesternSure® PREMIUM Chemiluminescent Substrate (Li-COR).

Table 1*. Primary antibodies used for immunoblot-analyses*

Primary antibody	
anti-Calnexin rb, Abcam, #ab22595 1:1000, 95 kDa	anti-CD9 rb, Abcam, #ab92726 1:1000, 25 kDa
anti-TSG101 rb, Abcam, #ab125011 1:1000, 45 kDa	anti-CD81 ms, SantaCruz, #cs-7637 1:500, 26 kDa

In addition EVs were characterized by flow cytometry measurement (CytoFlex S; Beckman Coulter). EVs (40 µg) incubated with APC anti-human CD81 (TAPA-1, BioLegend, #349509) and FITC anti CD9 monoclonal antibody (eBioSN4 (SN4 C3-3A2 Invitrogen, #11-0098-42) at 4°C for 1 h in the dark, centrifuged at 100.000 x g for 70 min using ultracentrifugation, resuspended in 100 µl PBS (Figure 3) and subjected to flow cytometry analyses.

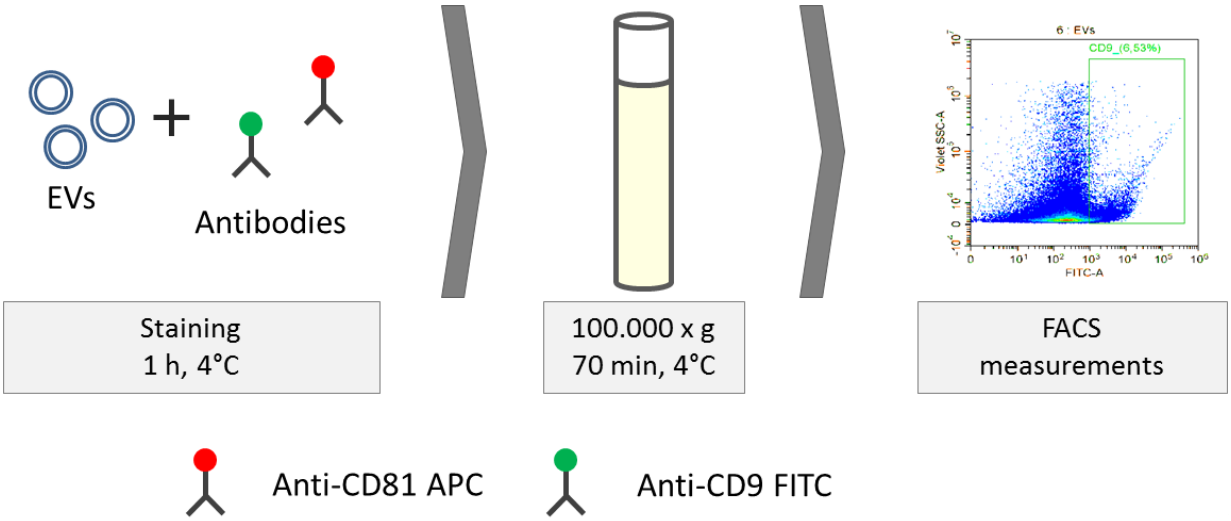

Figure 3. *Protocol of EV staining for flow cytometry analyses. The EVs were stained with the corresponding antibodies for 1 h at 4 °C, afterwards one washing step with ultracentrifugation was applied.*

For appropriate EV size gating, a Gigamix Standard (Biocytex, Marseilles, France) containing FITC-conjugated microparticles with a defined size ranging from 100 to 900 nm was applied. By this, a suitable gate for the EVs was defined according to the Gigamix-Standard (Figure 4) to cover all EVs in the size range from 100-900 nm.

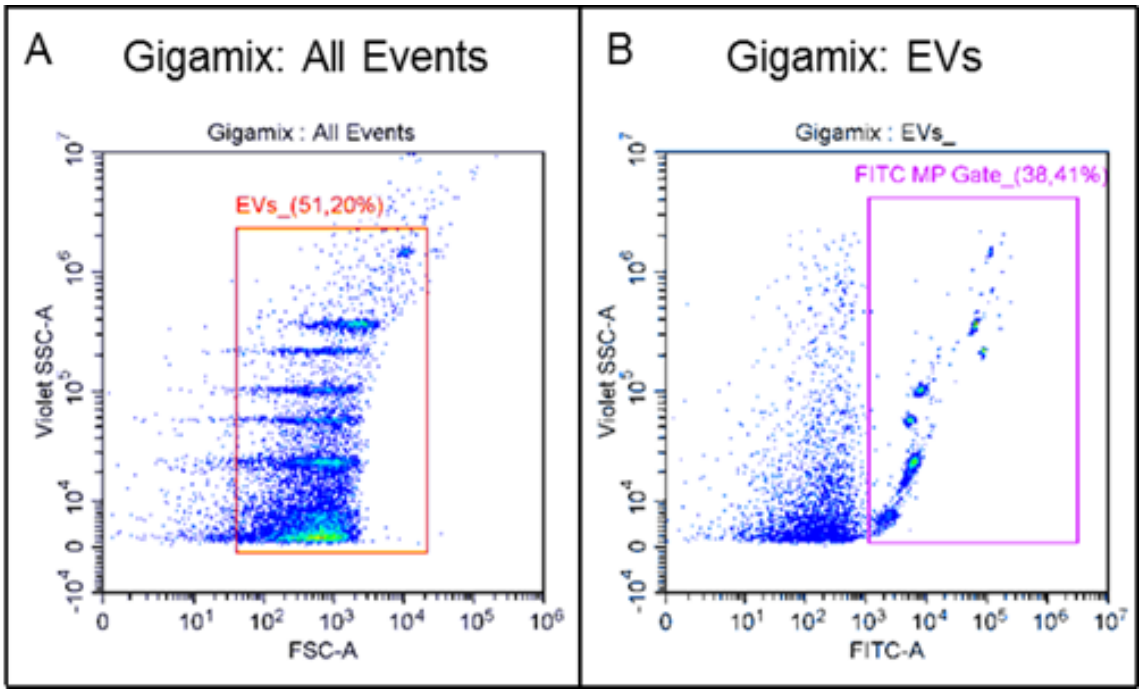

Figure 4*. Gating of EVs using Gigamix-Standard. The standard particles in the range of 100-900 nm were gated (A). Positive FITC events of the corresponding particles were gated (B).*

1.3 Results

Patients' and healthy donors' characteristics

In total, EVs were isolated, before and after irradiation with 2 x 2 Gy, from 12 leukaemic patients while the second sample of one patient was not assessable due to its bad health state. The median age of females was 42.8 years (n=5) and of males 53.4 years (n=7) (Table 2). 40 % of women suffer from acute myeloid leukaemia (AML) and 60 % from acute lymphoid leukaemia (ALL) (Table 2) while 85.7 % of all males suffer from AML and 14.3 % from ALL (Table 2). In addition, EVs were also isolated from 11 age matched healthy donors. The median age of female donors was 41.2 years (n=5) and of males 43.7 years (n=6).

Table 2. *Patients and healthy donors characteristics*

Donor	Sex	n	mean age/years	acute myeloid leukaemia/%	acute lymphoid leukaemia/%
Leukaemic patients	female	5	42.8±17	40.0	60.0
	male	7	53.3±8	85.7	14.3
Healthy donors	female	5	41.2±11	N/A	
	male	6	43.7±11		

Characterization of the EVs

As depicted in Figure 5, exemplary immunoblot analyses of EV markers TSG-101, CD81 and CD9 and the ER marker Calnexin of three patients before (d0) and after irradiation (d1) and of healthy donors revealed detection of CD9 in all patients and healthy donors and, as expected, lack of Calnexin detection in the EVs. By contrast, Tumor susceptibility gene 101 (TSG-101) detection was mainly restricted to patients derived EVs.

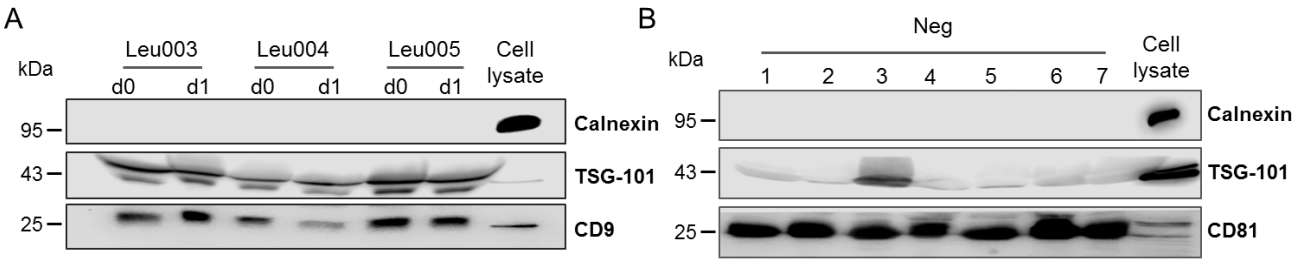

Figure 5. *EV marker detection and western immunoblot from EVs derived from leukaemic patients (Leu003-Leu005) before (d0) and after (d1) irradiation (A) and healthy donors (Neg1-7) (B).*

In flow cytometry analysis, we confirmed the positive signals for CD9 in healthy donors and leukaemic patients (Figure 6). In addition and in contrast to Western blot analyses, in patient-derived EVs a positive signal for CD81 was detected in both, leukaemic patients and healthy donors. Next, the EVs from human serum were distributed to the partners. In total, 40 µg per sample (leukaemic patients and healthy donors) were sent to HMGU to perform proteomic analysis (see chapter 2) and to Arraystar (Rockville, USA) to perform miRNA analyses (see chapter 3).

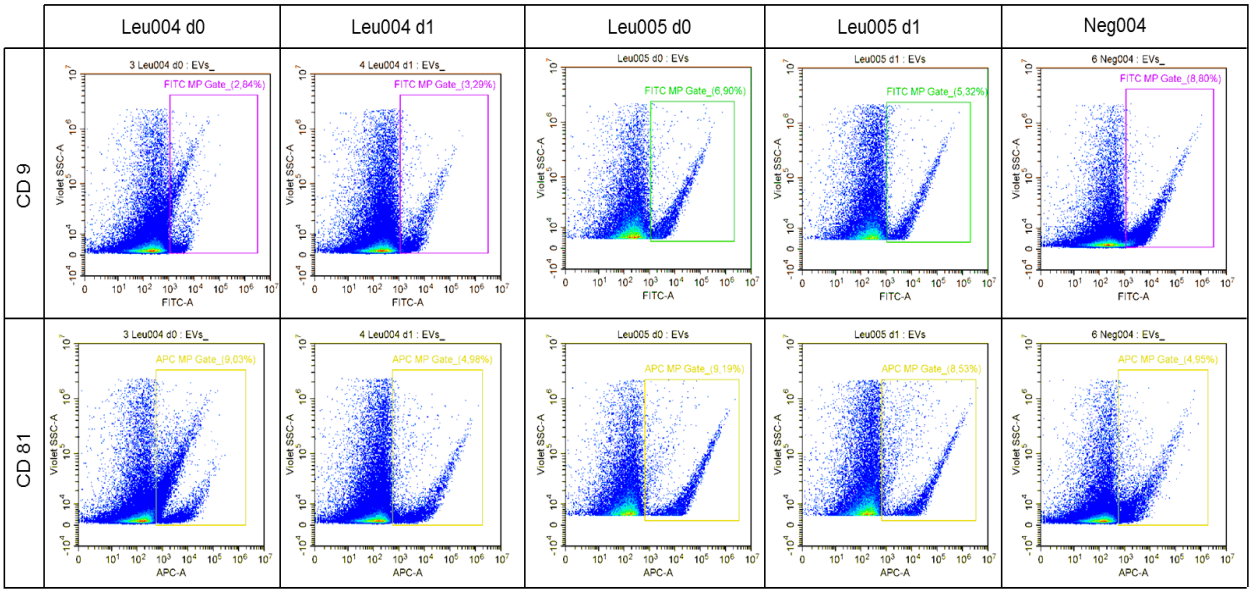

Figure 6. *EVs from leukaemic patients and healthy donors show positive signals for CD9 and CD81. Exemplary histograms from leukaemic patients Leu004 and Leu005 before (d0) and after (d1) irradiation and one healthy donor are given.*

1.4. Conclusion

The results of the Western blot and flow cytometry analysis indicate that isolation protocol by ultracentrifugation for EVs from serum was successful as detection of typical EV markers such as CD9, CD81 and TSG-101 was suitable in both leukaemic patients and healthy donors.

2. Proteomic analysis of EVs cargo from leukaemic patients and healthy donors

2.1 Method

EVs from healthy controls (n=6) and patients (n=5) with acute myeloid/lymphoid leukaemia were included in the study. Serum-derived EVs were isolated at GUF *via* ultracentrifugation (UC) as reported before. At HMGU samples were lysed with radioimmunoprecipitation buffer (RIPA) and trypsinized according to the in-house FASP protocol. Individual peptides were separated and identified using a high-performance liquid chromatography (LC) coupled to a mass spectrometer (MS) (QExactive™ HF, ThermoFisher). MS/MS raw files were analysed using Proteome Discoverer 2.2 software (ThermoFisher) and proteins were identified using a 1 % false discovery rate (FDR). For proteins identified with at least two unique peptides, significant deregulation was defined with a fold change of +1.3 and an adjusted p-value of < 0.05 (one-way ANOVA with Benjamin-Hochberg correction).

2.2. Results

In this study, 255 proteins were identified when 1 % FDR (false discovery rate) was applied, amongst which 199 proteins were identified with at least two unique peptides. A clustering in principal component analysis (PCA) for healthy controls was observed (Figure 7) based on intensities of the identified proteins.

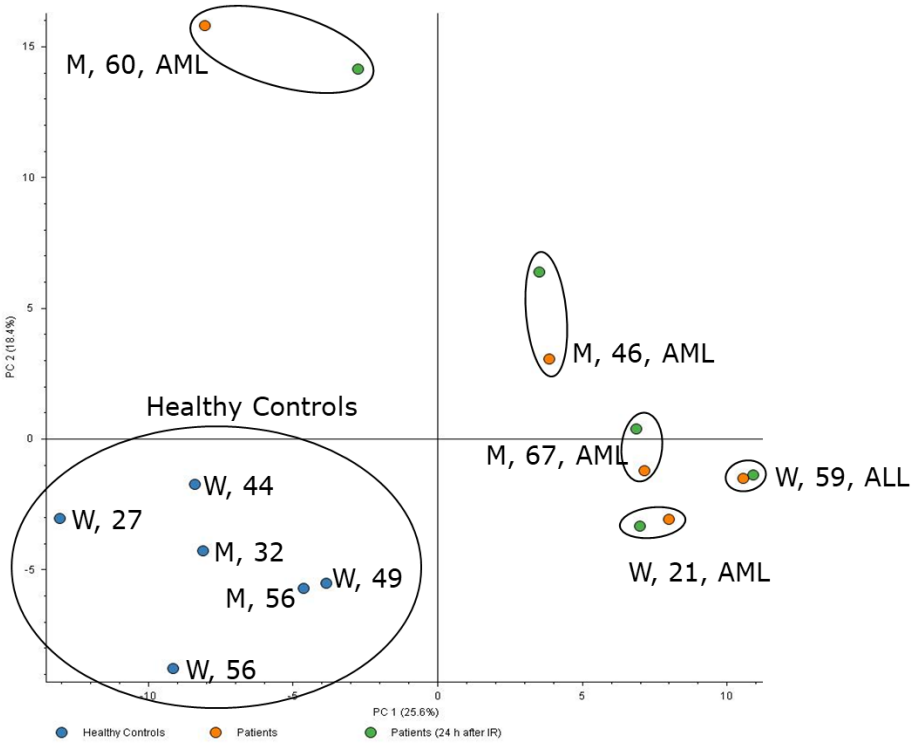

Figure 7. *Principal component analysis (PCA) based on all proteomic features of serum-derived EVs from healthy controls and leukemic patients. Clustering of healthy control samples (blue) is observed. The sex (M/W), age, and the type of leukaemia if relevant is indicated. The PCA coordinates for the same patient before (orange) and after exposure to IR (green) were not markedly changed, indicating only small alterations in the EV cargo due to irradiation.*

Protein abundances in patient EVs before exposure to IR were compared with healthy controls. Six downregulated and 21 upregulated proteins were observed in the patient EVs in comparison to the EVs from healthy donors (Supporting information 1).
The significantly deregulated proteins were subjected to an *in silico* enrichment analysis with the STRING web tool (https://string-db.org/). A clear clustering of all except two proteins was shown (Figure 8). A great majority (96%) of these proteins were involved in cellular defence (20/27), response to stress (22/27) or response to stimulus (26/27).

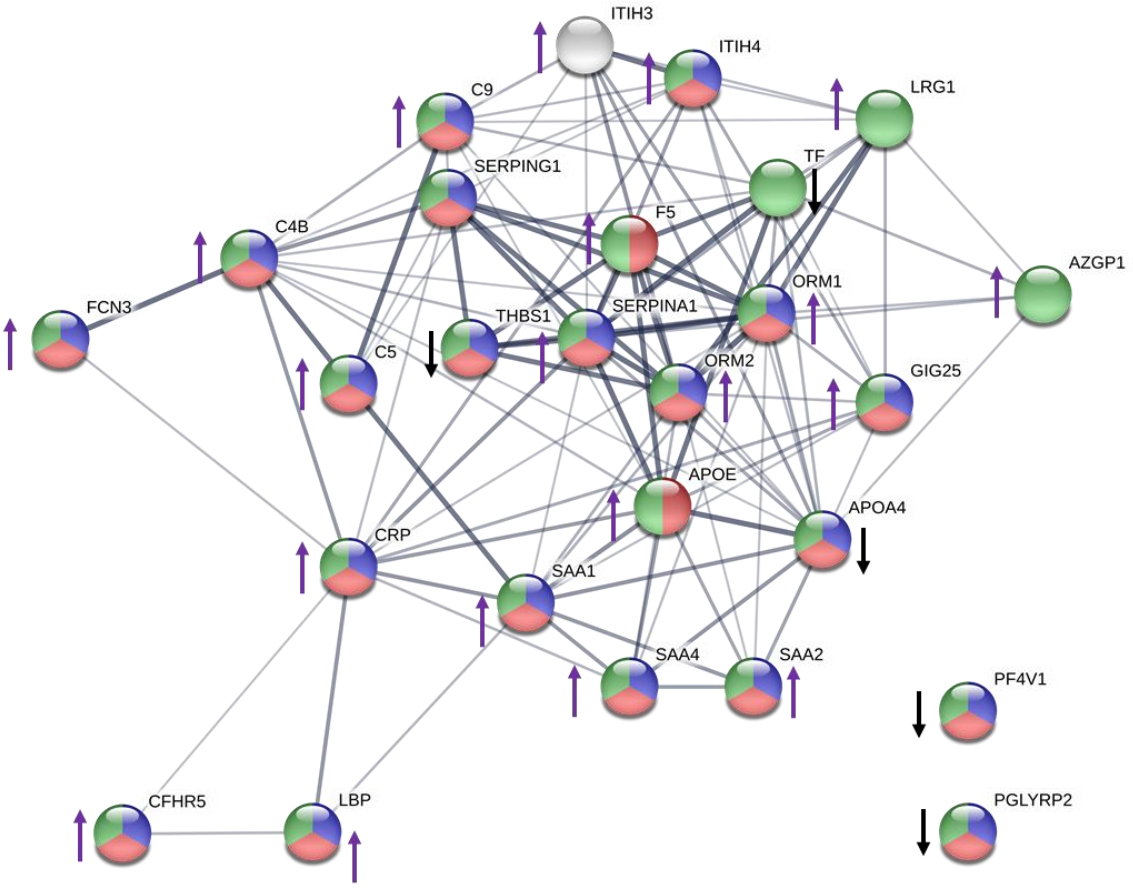

Figure 8. *Deregulated proteins identified in serum-EVs from leukemic patients (d0-EVs) compared to healthy controls. The proteins with most 'counts' in the gene set belonging to defence response (GO:0006952, FDR 1.15 × 10^{-16}, blue), response to stress (GO:0006950, FDR 1.15 × 10^{-11}, red) and response to stimulus (GO:0050896, FDR 1.11 × 10^{-7}, green) were present. Up- and downregulated proteins are marked with purple and black arrows, respectively.*

To evaluate radiation biomarkers in EVs, the protein abundances in patient EVs 24 h after irradiation (d1-EV) were compared with EVs of the same patient before irradiation (d0-EV). Fourteen proteins that were upregulated and 23 downregulated proteins (fold change ±1.3) in at least three out of five patients were subjected to *in silico* enrichment analysis (Figure 9 and Supporting Information 2). This analysis showed two distinct clusters of proteins (Figure 9). The big cluster consisted of 30 proteins and the small one of 5 proteins. Only two proteins had no interaction with any other deregulated protein. Also in this case most of the proteins belonged to cellular defence, stress response or response to stimulus as indicated in the Figure 9.

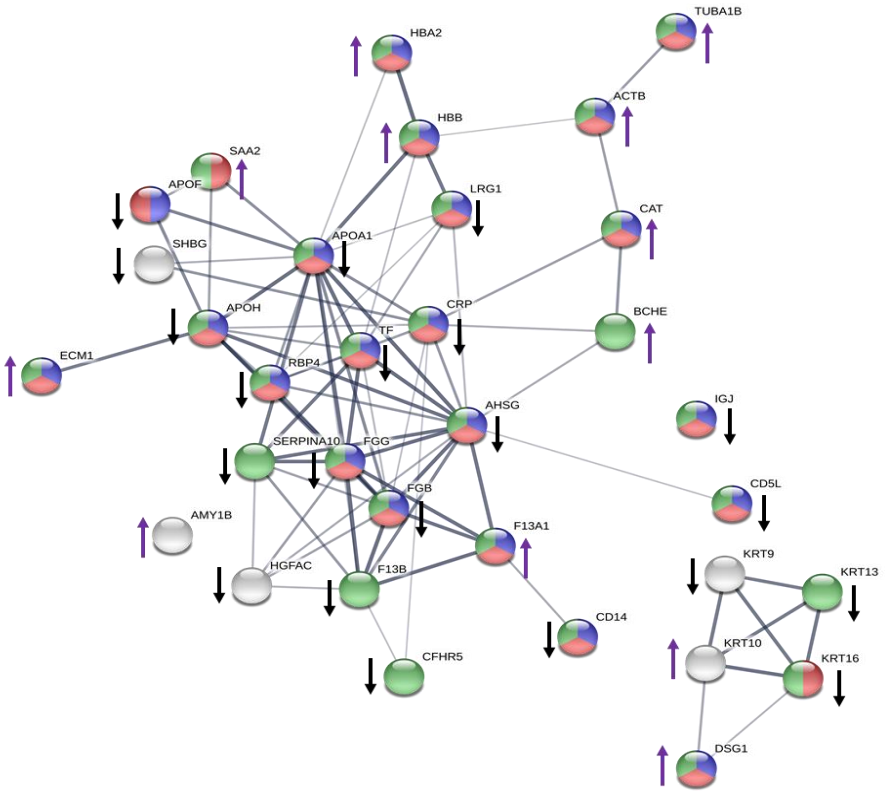

Figure 9. *Deregulated proteins observed in serum-EVs in at least three out of five patients after irradiation. The proteins with most 'counts' in gene set belonging to transport (GO:0006810, FDR 1.15 × 10^{-11}, blue), localization (GO:0051179, FDR 3.01 × 10^{-5}, red), and response to stimulus (GO:0050896, FDR 7.14 × 10^{-5}, green) were present. Up- and downregulated proteins are marked with purple and black arrows respectively.*

There were four proteins commonly deregulated in both serum-EVs from patients (d0-EV) compared to healthy controls and serum-EVs from patients after IR (d1-EV) compared to before radiation (d0-EV). These are shown in Table 3.

Table 3*. List of common deregulated proteins between serum-EVs from leukaemic patients compared to healthy controls and in the serum-EVs 24 h after IR in leukaemic patients.*

Accession	Protein Description	Deregulation	
		Patients-EV/ Healthy-EV	Patients (d1-EVs/d0-EVs)
P02787	Serotransferrin [OS=Homo sapiens]	Down	Down
P02750	Leucine-rich alpha-2-glycoprotein [OS=Homo sapiens]	Down	Up
Q9BXR6	Complement factor H-related protein 5 [OS=Homo sapiens]	Down	Up
P02741	C-reactive protein [OS=Homo sapiens]	Down	Up
P0DJI9	Serum amyloid A-2 protein [OS=Homo sapiens]	Up	Up

2.3. Discussion

In this work package, markers of radiation exposure in serum-derived EVs were investigated in humans. Serum-derived EVs, before and after total body irradiation, were analysed for their protein content. Although the healthy controls showed clustering independent of the age or sex in the PCA based on the EV protein intensities, the PCA showed no marked clustering between the leukemic patients. The reason for this is unknown and more samples are needed to investigate this. Accordingly, significantly deregulated proteins could not be identified when the EVs of all the patients (d0-EVs) were treated as one group and compared against d1-EVs of all patients. Instead, the EVs from the same patient before and after irradiation were compared. Only those proteins were considered deregulated that showed same direction of expression change in at least three out of five patients. Most of the deregulated proteins were involved in localisation, transport and response to stress.

In the murine study (Deliverable 9.95), we have identified proteins that were deregulated in serum-derived EVs after IR (0.1 Gy and 3 Gy total body irradiation). Upregulation of SAA2 (Serum amyloid A-2 protein) and downregulation of RBP4 (Retinol-binding protein 4), FGG (Fibrinogen gamma chain) and FGB (Fibrinogen beta chain) was observed in both human and murine studies. Fibrinogen is an acute-phase protein and plasma concentration of fibrinogen was found to increase after infection or inflammation[4,5]. It remains unclear why the levels of fibrinogens have decreased in the EVs in this study after radiation exposure. Similar to our study, serum amyloid A protein was observed to be increased in the plasma of mice 24 h after 4 Gy or 8 Gy of total body irradiation[6]. The levels returned to normal 48 h after irradiation. It should be investigated if the levels of SAA change in the EVs in the long run.

The EV cargo is often being investigated as potential biomarker and pharmacological target for various diseases. In this study, we have identified proteins that were deregulated in leukemic patients compared to healthy controls. The deregulated proteins clustered around plasma proteases, serum amyloids and glycoproteins. The deregulation of such proteins could serve as a possible biomarker for diagnosing leukaemia. However, it needs to be mentioned that for such a biomarker study a cohort with large study population is necessary.

3. miRNA analysis of EVs cargo from leukaemic patients and healthy donors

3.1 Introduction

MicroRNAs (miRNAs) cover a class of short single-stranded non-coding RNA with a length of 18 to 23 nucleotides. They play an important regulatory role in gene expression, especially in gene silencing. MiRNAs regulate gene expression highly specifically at the post-transcriptional level[7]. They constitute potential biomarkers for leukaemia, because they can be secreted into all kind of biofluids[8]. In addition to protein expression we aimed to characterize miRNA content in patient- and healthy control-derived EVs. Against this background, we aimed to unravel miRNA content in both patient- and healthy-donors derived EVs.

3.2 Method

The miRNA from EVs from healthy donors was isolated with the RNeasy Mini Kit (QIAGEN) and should be subjected to a nanoString nCounter assay (nanoString Technologies). However, these establishing steps revealed that miRNA concentration from human serum samples were too low for analyses with nCounter. As an alternative, we decided to change methodology to a next generation sequencing approach offered by a commercial company, Arraystar. According to the company's recommendation, miRNAs were isolated from blood samples of healthy donors and leukaemic patients before (d0) and after (d1) irradiation using miRNeasy Mini Kit (QIAGEN) designed to extract total RNA including microRNAs. The RNA yield covered 3.2 to 12 ng/µl.

3.3. Results

Samples covering in total three samples of healthy donors and three samples from patients with and without irradiation were transferred to the company and are being processed at present. Accordingly, a detailed analysis of the miRNA profile will be presented in the final report of the project.

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Supporting Information 1

List of deregulated proteins in serum-EVs in leukemic patients compared to healthy controls, their fold changes and adjusted p-values

Accession	STRING ID	Protein Description	Fold Change	Adj p-Value
P07996	TBHS1	Thrombospondin-1 [OS=Homo sapiens]	0.022	5.24E-04
P10720	PF4V1	Platelet factor 4 variant [OS=Homo sapiens]	0.035	2.05E-03
P01871		Immunoglobulin heavy constant mu [OS=Homo sapiens]	0.313	7.29E-03
P06727	APOA4	Apolipoprotein A-IV [OS=Homo sapiens]	0.44	4.52E-02
Q96PD5	PGLYRP2	Isoform 2 of N-acetylmuramoyl-L-alanine amidase [OS=Homo sapiens]	0.648	1.43E-02
P02787	TF	Serotransferrin [OS=Homo sapiens]	0.687	4.68E-03
Q14624	ITIH4	Inter-alpha-trypsin inhibitor heavy chain H4 [OS=Homo sapiens]	1.47	7.20E-03
P05155	SERPING1	Isoform 3 of Plasma protease C1 inhibitor [OS=Homo sapiens]	1.55	7.18E-03
P0C0L5	C4B	Complement C4-B [OS=Homo sapiens]	1.58	1.88E-02
P19652	ORM2	Alpha-1-acid glycoprotein 2 [OS=Homo sapiens]	1.77	1.63E-02
P02649	APOE	Apolipoprotein E [OS=Homo sapiens]	1.79	4.81E-03
P01009	SERPINA1	Alpha-1-antitrypsin [OS=Homo sapiens]	1.83	2.41E-02
P25311	AZGP1	Zinc-alpha-2-glycoprotein [OS=Homo sapiens]	1.85	7.20E-03
P01031	C5	Complement C5 [OS=Homo sapiens]	2.19	2.05E-03
O75636	FCN3	Ficolin-3 [OS=Homo sapiens]	2.20	1.12E-02
Q06033	ITIH3	Inter-alpha-trypsin inhibitor heavy chain H3 [OS=Homo sapiens]	2.22	2.32E-03
P35542	SAA4	Serum amyloid A-4 protein [OS=Homo sapiens]	2.26	7.29E-03
P02748	C9	Complement component C9 [OS=Homo sapiens]	2.36	5.24E-04
P01011	GIG25	Alpha-1-antichymotrypsin [OS=Homo sapiens]	2.52	2.54E-04
P02763	ORM1	Alpha-1-acid glycoprotein 1 [OS=Homo sapiens]	2.74	7.07E-04
P12259	F5	Coagulation factor V [OS=Homo sapiens]	3.49	2.78E-03
P02750	LRG1	Leucine-rich alpha-2-glycoprotein [OS=Homo sapiens]	3.95	7.07E-04
Q9BXR6	CFHR5	Complement factor H-related protein 5 [OS=Homo sapiens]	4.01	7.75E-04
P18428	LBP	Lipopolysaccharide-binding protein [OS=Homo sapiens]	4.68	1.19E-03
P02741	CRP	C-reactive protein [OS=Homo sapiens]	100	5.68E-05
P0DJI8	SAA1	Serum amyloid A-1 protein [OS=Homo sapiens]	100	6.82E-04
P0DJI9	SAA2	Serum amyloid A-2 protein [OS=Homo sapiens]	100	5.68E-05

Supporting Information 2

List of deregulated proteins identified in serum-EVs in at least three out of five leukemic patients after irradiation

Accession	STRING ID	Protein Description	Fold Change				
			Leu003	Leu004	Leu005	Leu006	Leu007
P02765	AHSG	Alpha-2-HS-glycoprotein [OS=Homo sapiens]	0.345	0.784	0.442	0.669	0.484
O43866	CD5L	CD5 antigen-like [OS=Homo sapiens]	0.549	0.496	0.468	0.688	0.958
P05160	F13B	Coagulation factor XIII B chain [OS=Homo sapiens]	0.738	0.694	0.422	0.559	1.093
P01817		Immunoglobulin heavy variable 2-5 [OS=Homo sapiens]	0.706	1.086	0.583	0.0100	0.0100
P04278	SHBG	Sex hormone-binding globulin [OS=Homo sapiens]	0.596	1.24	0.242	0.718	0.224
P02749	APOH	Beta-2-glycoprotein 1 [OS=Homo sapiens]	0.591	0.487	0.536	0.698	1.40
P13646	KRT13	Keratin, type I cytoskeletal 13 [OS=Homo sapiens]	0.0100	0.063	100	0.575	0.0370
P01591	IGJ	Immunoglobulin J chain [OS=Homo sapiens]	0.134	0.667	0.692	1.44	0.197
P02787	TF	Serotransferrin [OS=Homo sapiens]	0.815	0.769	0.692	0.708	1.03
P02647	APOA1	Apolipoprotein A-I [OS=Homo sapiens]	0.601	1.08	0.721	0.852	0.736
P02750	LRG1	Leucine-rich alpha-2-glycoprotein [OS=Homo sapiens]	0.677	0.642	0.879	0.873	0.664
P02753	RBP4	Retinol-binding protein 4 [OS=Homo sapiens]	0.867	0.623	0.251	0.419	0.994
P02741	CRP	C-reactive protein [OS=Homo sapiens]	0.762	0.782	0.999	0.688	0.557
Q13790	APOF	Apolipoprotein F [OS=Homo sapiens]	0.595	1.09	0.570	0.784	0.585
Q9UK55	SERPINA10	Protein Z-dependent protease inhibitor [OS=Homo sapiens]	0.728	0.723	1.16	0.678	0.790
Q04756	HGFAC	Hepatocyte growtH factor activator [OS=Homo sapiens]	0.837	0.366	0.463	0.606	1.12
Q9BXR6	CFHR5	Complement factor H-related protein 5 [OS=Homo sapiens]	0.610	1.22	0.691	0.717	0.886
P06276	BCHE	cholinesterase [OS=Homo sapiens]	0.847	0.188	0.221	0.619	1.53
P08571	CD14	Monocyte differentiation antigen CD14 [OS=Homo sapiens]	0.0100	0.668	0.010	0.960	1.85

P35527	KRT9	Keratin, type I cytoskeletal 9 [OS=Homo sapiens]	1.34	0.499	12.6	0.497	0.421
P08779	KRT16	Keratin, type I cytoskeletal 16 [OS=Homo sapiens]	0.0100	1.79	100	0.582	0.111
P02679	FGG	Fibrinogen gamma chain [OS=Homo sapiens]	0.280	53.3	0.551	1.67	0.647
P02675	FGB	Fibrinogen beta chain [OS=Homo sapiens]	0.268	26.0	0.547	1.50	0.729
P04745	AMY1B	alpha-amylase 1 [OS=Homo sapiens]	2.63	15.8	3.33	17.8	22.6
P01701		immunoglobulin lambda variable 1-51 [OS=Homo sapiens]	1.44	0.531	1.56	1.99	1.92
P00488	F13A1	Coagulation factor XIII A chain [OS=Homo sapiens]	3.84	6.48	100	7.55	1.09
P60709	ACTB	Actin, cytoplasmic 1 [OS=Homo sapiens]	1.77	0.636	2.18	2.72	0.308
P13645	KT10	Keratin, type I cytoskeletal 10 [OS=Homo sapiens]	1.31	0.65	2.12	1.50	0.87
P0DJI9	SAA2	Serum amyloid A-2 protein [OS=Homo sapiens]	1.68	0.194	2.55	1.61	1.10
P68871	HBB	Haemoglobin subunit beta [OS=Homo sapiens]	1.62	1.20	1.56	3.33	0.617
P69905	HBA2	Haemoglobin subunit alpha [OS=Homo sapiens]	1.51	1.13	1.62	3.54	0.737
P01700	-	Immunoglobulin lambda variable 1-47 [OS=Homo sapiens]	0.883	0.489	1.47	1.73	1.44
P01717	-	Immunoglobulin lambda variable 3-25 [OS=Homo sapiens]	0.77	0.397	1.45	1.64	1.34
P68363	TUBA1B	Tubulin alpha-1B chain [OS=Homo sapiens]	4.54	0.112	2.00	0.862	2.02
Q02413	DSG1	Desmoglein-1 [OS=Homo sapiens]	4.36	0.682	8.13	1.42	1.23
Q16610	ECM1	Isoform 4 of Extracellular matrix protein 1 [OS=Homo sapiens]	1.17	9.50	1.07	1.42	1.31
P04040	CAT	Catalase [OS=Homo sapiens]	2.34	1.13	2.51	3.98	0.853