RADIATION RESEARCH **182**, 299–309 (2014)
0033-7587/14 $15.00
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DOI: 10.1667/RR13645.1

Independent Validation of Candidate Genes Identified after a Whole Genome Screening on Mayak Workers Exposed to Prolonged Occupational Radiation

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Abend, M., Azizova, T., Müller, K., Dörr, H., Senf, S., Kreppel, H., Rusinova, G., Glazkova, I., Vyazovskaya, N., Unger, K. and Meineke, V. Independent Validation of Candidate Genes Identified after a Whole Genome Screening on Mayak Workers Exposed to Prolonged Occupational Radiation. *Radiat. Res*. 182, 299–309 (2014).

We evaluated gene expression in the peripheral blood of Mayak workers in relationship to occupational chronic exposure to identify permanent post-exposure signatures. The Mayak workers had experienced either a combined exposure to incorporated ^{239}Pu and external gamma rays (n = 82) or exposure to external gamma rays (n = 18). Fifty unexposed individuals served as controls. Peripheral blood was collected and then the RNA was isolated, converting it into cDNA and stored at −20°C. In a previous study at stage I, we screened the mRNA and microRNA transcriptome using 40 of the 150 samples and identified 95 mRNAs and 45 microRNAs. In stage II of this study, we now validated our 140 candidate genes using the qRT-PCR technique for the remaining 92 blood samples (18 samples were lost due to methodological reasons). We analyzed associations of normalized gene expression values in linear models separately for both exposure types (continuous and categorical scales) and adjusted for exposure age as well as stratified by gender. After further adjustment for confounders such as chronic non-cancer diseases or age at biosampling, mostly binary (on/off) dose-to-gene relationships were found for 15 mRNAs and 15 microRNAs, of which 8 mRNAs and 6 microRNAs remained significant after Bonferroni correction. Almost all of them were associated with plutonium incorporation and gender. Our study provides mRNA and microRNA gene expression changes dependent on the exposure type and gender, which occur and seem to persist after chronic radiation exposures supporting the concept of permanent post-exposure signatures. © 2014 by Radiation Research Society

INTRODUCTION

There have been long and ongoing discussions about whether past exposure to ionizing radiation leaves a unique and permanent signature in the genome (*1*). Identification of such a signature would strongly impact epidemiological studies, since low-dose epidemiologic risk is greatly hindered by current limitations to estimate past radiation exposure on an individual basis (*2*). Recent studies using the chromosome labeling techniques, mBand and mFISH, suggested that interchromosomal aberrations (*3, 4*) and complex chromosome aberrations (*2*) in lymphocytes persist in individuals many years after occupational exposure to densely ionizing radiation. These chromosome labeling techniques have been applied to healthy former nuclear weapon production workers who had been occupationally exposed since 1949 at the Mayak Production Association (PA) near Ozyorsk, Russia (*5, 6*). These radiation workers were employed either at plutonium manufacturing/processing facilities or at a nuclear reactor facility. Unlike the reactor workers, the plutonium workers were exposed to densely ionizing alpha particles due to plutonium inhalation.

Based on previous studies of Chernobyl thyroid cancer biopsies, we found evidence that long-lasting dose-related changes of gene expression might be present in thyroid tissue, which was potentially indicative of early events in the multistep process of radiation carcinogenesis (*7, 8*).

Encouraged by these findings we decided in favor of a two-stage study design examining gene expression in blood samples derived from 150 Mayak workers exposed to either external gamma rays only (n = 18) or combined incorporated ^{239}Pu and external gamma rays (n = 82).

Editor's note. The online version of this article (DOI: 10.1667/RR13645.1) contains supplementary information that is available to all authorized users.

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Inclusion/exclusion criteria

	external γ-ray only	external γ-ray & internal Pu	N
• # exposed Ozyorsk residents	3,803	5,584	9,387
• total dose >0.5 Gy/>1.01 Gy + >0.7 kBq[1]	1,514	579	2,093 (100%)
• known vital status (May-June 2011)	1,469	579	2,048 (97.8%)
• alive & living in Ozyorsk during the study	301	153	454 (21.7%)
• # exposed residents involved in our study	18	82	**100 (4.8%)**
• # unexposed residents involved in our study			**+ 50**
			150

Phase I (two stage study design)

1. random split sample set: Phase I 40[2] / Phase II 110
2. screening set with 4x10 RNA samples comprising an unexposed (control) group and 3 exposure groups[3]
3. examining
   - mRNA transcriptome (whole genome microarray, 19,596 genes)
     → ~ 500 mRNA (335 genes)[4]
     → gene enrichment analysis (PANTHER)
     → 95 mRNA for phase II
   - microRNA transcriptome (qRT-PCR, ~ 667 microRNA)
     → 45 microRNA[5] for phase II

Phase II (two stage study design)

1. independent validation set with 92 samples[6]
2. employing 6 statistical models separately for each exposure type and gene[7]
3. adjusting significantly associated candidates (25 mRNA, 20 microRNA) with 26 confounders → 15 mRNA and 15 microRNA survived
4. perform non parametric Kruskal Wallis test (3df) on the surviving candidate genes
5. examine mRNA-microRNA relationship
6. analytically determine association of final mRNA/microRNA candidates with blood cell counts

Laboratory Technique Validation

Comparison of phase I whole genome microarray data (40 samples) with Phase II qRT-PCR data (92 samples)

FIG. 1. Flow diagram depicting included samples, split study design, gene expression measurements and bioinformatics with focus on phase II (black letters). Chapters with gray letters (inclusion/exclusion criteria, phase I) refer to previous analysis on the same cohort.

In our previous stage I analysis of that study, we screened the whole mRNA and large parts of the microRNA transcriptome using 40 of our 150 samples and identified 95 candidate genes (mRNAs) and 45 candidate microRNAs (*9*). These genes were then utilized in stage II, and were validated using a more sensitive and specific approach (qRT-PCR) compared to the previously employed microarray analyses. In addition, an independent analysis was performed of the remaining 92 blood samples (18 samples were lost due to methodological reasons). Study results from stage II are presented here.

MATERIALS AND METHODS

Study Population

The individuals in this study population were occupationally exposed to ionizing radiation from 1949 onward at the Mayak Production Association near Ozyorsk, Russia (*5, 6*). The plutonium workers were exposed to densely ionizing alpha particles due to plutonium inhalation and were also exposed to sparsely ionizing gamma rays. The reactor workers were not exposed to plutonium, but were exposed to sparsely ionizing gamma rays. Radiochemical workers were also exposed to a variety of chemical mutagens. The control group consisted of Ozyorsk residents who were never exposed occupationally and never involved in any cleanup operations after a radiation accident and who never lived in contaminated areas. Biophysical examinations of the unexposed individuals (controls) revealed no plutonium (Pu) in their bodies. This article reports on our study of 150 individuals from the Mayak radiation worker cohort, of whom 18 were exposed to external gamma rays only and 82 were exposed both to alpha particles due to plutonium incorporation and external gamma rays. The remaining 50 individuals, who served as controls, were unexposed Ozyorsk residents (Fig. 1). Only those Ozyorsk residents with known vital status (May–June 2011) and who were alive in 2011 were included in our study.

Total body-doses were >0.5 Gy for individuals only exposed to external gamma rays. For individuals with combined occupational

exposures, total whole-body doses of external gamma rays had to exceed 1.01 Gy and the plutonium body burden was >0.7 kBq (Fig. 1). Blood samples were taken in 2011. We lost 18 samples (including 4 samples from individuals who were deceased within 6 months after blood sampling or who had a malignant cancer) due to malfunctioning consumables (mirVana Kit). Forty samples were used for screening purposes to search for candidate genes in phase I, leaving 92 blood samples that were eligible for validation in phase II. In addition to individual occupational dose estimates, individual data were available regarding age at exposure, age at biosampling, demographic data (education, ethnicity, birth date, gender), social habits (smoking and alcohol), data related to the health status [diastolic/systolic blood pressure, body mass index (BMI)], diagnosis of benign (mostly skin) tumors as well as chronic non-cancer diseases (chronic radiation syndrome, atherosclerosis, fibroadenoma of breast, cholelithiasis, chronic gastritis, chronic pulmonary disease, diabetes, endocrine related diseases, hypertension, ischemic heart disease, kidney disease, pancreatic disease, Parkinson's disease, prostatic hyperplasia, thyroid diseases, gastric ulcer and venous diseases), a total of 26 variables with potential impact on our dose-to-gene relationships (confounders were identified). The following diseases, which had frequencies <10 in our groups, were excluded from the analysis: acute respiratory disease (n = 1); cerebrovascular disease (n = 3); liver cirrhosis (n = 9); connective tissue diseases (n = 7); infectious disease (n = 3); nervous disease (n = 2); rheumatic disease (n = 1); skin diseases (n = 8); tuberculosis (n = 0); and viral hepatitis (n = 0). Additionally, organ-specific doses of medical diagnostic radiation exposures were provided for our exposed individuals (only) as well as blood cell counts at the time of biosampling. The study was approved by the ''Observation Council SUBI'' (Institutional Review Board, SUBI) and is responsible for the control of current ethical norms. An informed consent to participate in the study, approved by the IRB SUBI, was signed by each participant.

Dosimetry

Individual absorbed doses from internal alpha particles and external gamma rays to the red bone marrow (RBM) were estimated for each of the studied individuals. Estimates of plutonium exposure were based on an average of nine urine sample measurements (*10–14*) and organ doses were calculated based on biokinetic models (*12, 15–18*). Unlike the gamma-ray exposure, which terminated at the end of each individual's work period, a fraction of the plutonium exposure occurred subsequently, because of long-term retention of a fraction of the plutonium intake. For the plutonium workers studied here, an average of 50% of the plutonium dose had been deposited in the bone marrow after 1983, 25% was deposited after 1993 and 8% was deposited after 1998, as estimated with the dosimetry system described above. Data on gamma-ray exposures from external sources were based primarily on film badge data (*19*) and could be confirmed by experimental electron paramagnetic resonance measurements in tooth enamel of 62 workers at Mayak PA (*20*). The reactor workers also had very low exposure to neutron radiation: the maximum neutron dose to the bone marrow was estimated to be less than 0.3% of the gamma-ray dose.

RNA Extraction and Quality Control

Whole blood samples (2.5 ml) were taken using the PAXgene Blood RNA system (BD Diagnostics, PreAnalytiX GmbH, Hombrechtikon, Switzerland) and processed accordingly [for details see ref. (*9*)].

Phase I Screening: Whole Genome Microarray and Micro-RNA Experiments

In a previous study we performed a whole genome screening for differentially expressed genes [protein coding mRNAs, (*9*)] using 4 × 10 RNA samples hybridized to the Agilent oligo microarray GE 8 × 60K (Agilent Technologies, Waldbronn, Germany) on four groups consisting of one unexposed (control) group and three exposure groups with the latter reflecting the strongest contrast of both occupational exposure types to our controls [Fig. 1, for details see ref. (*9*)]. We used the nonparametric Kruskal-Wallis test (KW P value) to compare gene expression across three dose groups and the control and finally identified 95 gene candidates for validation by qRT-PCR in phase II (Fig. 1). Only genes that were: 1. detectable in at least 50% of RNA specimens; 2. showed a significance unadjusted $P \leq 0.05$ using the Kruskal-Wallis test; 3. revealed greater than or equal to twofold gene expression difference among compared groups; and 4. identified by an inventoried TaqMan® assay (to run qRT-PCR analysis) were utilized.

We evaluated microRNA gene expression by qRT-PCR (TaqMan primer probe assays) in the same 4 × 10 RNA samples using a low-density array [(LDA) type A and B, Life Technologies, Darmstadt, Germany]. Altogether 667 microRNA could be analyzed employing two 384-well LDAs (LDA, type A and B) as described earlier [Fig. 1, for details see ref. (*9*)]. Altogether 45 microRNA candidates were detectable in at least 50% of RNA specimens and revealed significant exposure-to-gene associations and all of them were moved to phase II for validation by qRT-PCR (Fig. 1).

Phase II: Validation of Phase I Candidate Genes

In the present study to validate the 95 candidate genes (mRNA) we employed a custom made LDA. For validation of our 45 microRNA we used the same microRNA LDA (type A and B) which was employed earlier in phase I, except that we restricted our analysis to the 45 microRNAs which appeared significantly associated with dose and did not analyze the other 621 microRNA species. Hence, all data were generated employing different LDA qRT-PCR platforms. Both mRNA and microRNA were examined together (140 genes total). Gene values had to be normally distributed for further analysis, which could be accomplished after log or Box-Cox transformation if needed. We compared gene expression across three dose categories for internal plutonium (0–0.055 Gy; >0.055–0.085 Gy; >0.085) and external gamma-ray exposure (0–0.5 Gy; >0.5–1 Gy; >1 Gy) with cutoff points approximately corresponding to tertiles of dose distribution among cases. Dose-to-gene associations were measured in linear models separately for each exposure type, using dose on a linear scale. Additionally these models were adjusted for age at exposure (except when using unexposed as the reference group) and stratified by gender. This approach enabled us to performing completely separate analyses for males and females. For those genes that were significantly associated with both exposure types, we re-ran the analysis and added both exposures into the same model. Using the linear models we calculated a 1 degree of freedom (*df*) P value per dose category, a 3 *df* P value over all four groups and a 2 *df* P value to examine a dose-to-gene relationship among the exposure groups only. Then we examined whether significant dose-to-gene relationships remained considering potential confounders. We added our 26 confounders separately to all models and for each exposure type and analyzed whether or not the dose-to-gene associations still existed. When adding confounding factors to our ''basic'' models we found that: 1. Confounders (e.g., age at blood sampling, smoking, alcohol, BMI) were not significantly associated with our models and, therefore, not included into the models; 2. Confounders were significantly associated with our models but significant exposure-to-gene associations became insignificant. In this case the gene was excluded from the further analysis, thus reducing the number of candidate mRNAs and miRNAs after controlling for potential confounder; 3. Confounders were significantly associated with our models (e.g., thyroid disease) and even improved the already significant exposure-to-gene associations. In this case we stayed with the ''basic'' model as well; and 4. Occasionally, confounders appeared significantly associated with and marginally

weakened the still significant exposure-to-gene relationship. For clarity, we followed the "basic" model. Dose-to-gene relationships still present after this procedure (the 1 *df* and 3 *df P* values had to remain significant during the procedure) were confirmed using the nonparametric Kruskal-Wallis test over all 4 groups (KW, 3 *df P* value).

In a final step we calculated the Pearson correlation coefficient and examined for significant associations between the genes and blood cell counts on the day of biosampling using the linear regression analysis. Blood cell counts associated with gene expression of our candidate genes comprised an absolute number of leucocytes, basophil/eosinophil/neutrophil granulocytes (separately for band form and segmented) in percent as well as lymphocytes and monocytes in percent.

All data analyses were performed in SAS (SAS 9.2, 2010; SAS Institute, Cary, NC).

Finally, we compared the mean differential gene expression (exposed vs. unexposed) of whole genome microarray data from phase I individuals with the mean differential gene expression of qRT-PCR data from phase II individuals for the same genes to check for the reliability of the methods employed. The mean differential gene expression in phases I and II data (used as reference) showed an overall agreement of 80.8% with a 19.2% false positive rate supporting the reliability of the methods.

Bioinformatic Analysis

We also examined the miR-mRNA target-relationships of the genes passing adjustments for confounding variables, by utilizing the well established TargetScan database (http://www.targetscan.org/) a common standard for miRNA target predictions.

RESULTS

Characteristics of Our Cohort

As previously shown, unexposed groups (control) and exposed individuals (summarized into one group due to low frequency of Mayak workers exposed to external gamma rays only) show considerable differences based on gender [control: 70% females; exposed: 39% females (Table 1)]. Female predominance in the control group also accounted for a number of differences in several areas, including: social behavior (control group members did smoke and drink less often); frequency differences in chronic non-cancer diseases such as thyroid diseases (19 vs. 69%, controls/exposed); atherosclerosis (26 vs. 62%, controls/exposed); and diabetes (7 vs. 20%, controls/exposed). Regarding BMI, systolic blood pressure, ethnicity or age at blood sampling (median: 78 vs. 80 years, controls/exposed) we observed no statistical difference between both groups. Mayak workers suffering from chronic radiation sickness were found in the exposed group only.

The equivalent red bone marrow dose of mean occupational external gamma-ray exposure (over all exposure groups) was 1.4 Gy (SD $\pm$0.51), ranging between 0.39 Gy and 3.1 Gy. The mean absorbed red bone marrow dose from internal alpha radiation due to plutonium intake was 0.11 Gy (SD $\pm$0.13), ranging between 0.004–1.01 Gy. To calculate the equivalent dose, these values have to be multiplied by an RBE (radiobiological efficiency) of 20 for alpha particles (*21*). Among our Mayak workers we observed comparable ages of first exposure to either external gamma rays (median: 21 years; range: 17–31 years) or internal plutonium particles [median: 22 years; range: 17–35 years (Table 1)].

Dose-to-Gene Relationships

Only 45 of the 95 candidate mRNAs from phase I showed gene expression values in $\geq$50% of our samples. Of these we log (n = 7) and Box-Cox (n = 5) transformed 12 genes to fulfill the criteria of normal distribution for all of our mRNAs using the Kolmogorov-Smirnov test. We also examined all 45 microRNA species from phase I. Additionally we initialized 5 variables for those microRNAs showing significant differences in the number of detectable gene values over the 4 groups [for details see ref. (*9*)]. Missing Ct values were arbitrarily set to 30 representing the upper limit of the linear dynamic range of our microRNA qRT-PCR measurements. Normal distribution of these 50 variables was accomplished after log (n = 4) and Box-Cox (n = 5) transformation for 9 of our 50 microRNAs and confirmed using the Kolmogorov-Smirnov test. Therefore, a total of 95 variables (45 mRNA and 50 microRNA) were entered into our analysis.

We found 25 mRNA and 20 microRNA significantly associated with either internal alpha-particle exposure due to incorporated plutonium or external gamma-ray exposure, a number which declined to 15 mRNA and 15 microRNAs after adjusting our models for confounding variables (Table 2). None of the five additionally initialized microRNA variables appeared to be significantly associated with both exposure types. When adjusting *P* values for multiple comparisons utilizing Bonferroni correction, a total of 8 mRNAs and 6 microRNAs had significant *P* values with $P \leq 0.001$ for mRNAs (0.05/50) and miRNAs [0.05/45 (Table 2)].

Comparisons of the mRNA and microRNA transcriptomes revealed a similar preference of dose-to-gene associations with internal alpha-particle exposure due to incorporated plutonium (transcriptional 93.3%; post-transcriptional 86.7%), but linear dose-to-gene relationships were found in 21/19% only (Fig. 2, Tables 2 and 3). Binary dose-to-gene relationships for genes that were similarly up/downregulated over all three dose categories relative to the unexposed controls [on/off pattern (Fig. 2)] predominated with 74% at the mRNA transcriptome over 38% at the microRNA transcriptome. Several genes (Table 2) were selected to visualize these different dose-to-gene relationships in Fig. 2. The number of up/downregulated genes, fold differences in gene expression (reference: unexposed group) and its association with gender showed an inverse pattern: at the mRNA transcriptome we primarily (89.5%) observed less than or equal to twofold gene expression differences and 94.7% upregulated genes mostly in males (86.7%). At the microRNA transcriptome we found greater

TABLE 1
Group Characteristics of the Unexposed Controls (27 Individuals) and all Mayak Workers Exposed to One or Both Types of Exposures Summarized into One Exposure Group (65 Individuals)

	n (92)	Control (27)		Exposed (65)		χ^2
		n	Percentage	n	Percentage	(P value)
Gender						
men	48	8	29.6	40	61.5	
women	44	19	70.37	25	38.5	0.005
Ethnicity						
Russian	78	21	77.8	57	87.7	
others	14	6	22.2	8	12.3	0.2
Age at blood sampling (years)						
<77	30	11	40.7	19	29.2	
77–82	31	7	25.9	24	36.9	
>82	31	9	33.3	22	33.9	0.5
median	78.0			80.0		
range	63–92			69–91		
Age at first external γ-ray exposure (years)						
17–19				19	29.2	
>19–22				20	30.8	
>22				26	40.0	nd
none				27		
median				21		
range				17–31		
Age at first internal plutonium exposure (years)						
17–19				17	27.9	
>19–22				18	29.5	
>22				26	42.6	nd
none				31		
median				22		
range				17–35		
Smoker						
never	59	24	92.3	35	53.9	
ever	32	2	7.7	30	46.2	0.0005
unknown	1					
Alcohol						
never	34	17	65.4	17	26.2	
ever	57	9	34.6	48	73.9	0.0005
unknown	1					
BMI						
≤26	32	12	44.44	20	30.8	
>26	60	15	55.56	45	69.2	0.2
median		27		28.1		
range		22.6–38.1		18.2–35.7		
Systolic blood pressure (mmHg)						
<140	50	20	74.07	30	46.2	
≥140	42	7	25.9	35	53.9	0.01
median		140.0		150.0		
range		95–180		110–185		
Chronic radiation sickness						
never	62			35	53.9	
ever	30			30	46.2	<0.0001
Thyroid disease						
never	42	22	81.5	20	30.8	
ever	50	5	18.5	45	69.2	<0.0001
Atherosclerosis						
never	45	20	74.1	25	38.5	
ever	47	7	25.9	40	61.5	0.002
Diabetes						
never	77	25	92.59	52	80.0	
ever	15	2	7.4	13	20.0	0.1

Notes. Categories often follow a separation into groups to obtain equal size (e.g., age at blood sampling) or reflect medical significance, e.g., normal ($<$140 mmHg) vs. increased ($\geq$140 mmHg) systolic blood pressure or normal BMI ($\leq$26) vs. preobesity and obesity (BMI $>$26). Significant differences in frequency distributions among groups are calculated employing a χ^2 statistic.

TABLE 2
Significant Dose-to-Gene Relationships of 15 mRNAs and 15 microRNAs Remaining after Adjustments with Known Confounders are Shown/Ordered Separately by Exposure Type and Gender

			Dose linear		Dose categorical													KW
					Reference		I			II			III			3 *df*	2 *df*	3 *df*
Gene symbol	Exposure	Strata	n	*P*	n	FC	n	FC	*P*	n	FC	*P*	n	FC	*P*	*P*	*P*	*P*
mRNA Transcriptome (n = 15)																		
ADAM15	internal plutonium	male	46	0.2	10	1	7	1.9	0.0001*	14	1.4	0.02	15	1.2	0.2	0.003	0.03	0.01
		female	37	1.0	13	0	4	1.0	0.8	11	0.8	0.1	9	1.1	0.7	0.3	0.1	0.3
AGAP4,6,8	internal plutonium	male	45	0.1	10	1	7	1.5	0.009	13	1.4	0.01	15	1.2	0.3	0.03	0.1	0.05
ATF6B	internal plutonium	male	45	1.0	10	1	7	1.6	0.002	13	1.5	0.004	15	1.4	0.008	0.01	0.9	0.007
LOC731275	internal plutonium	male	46	0.7	10	1	7	1.9	0.004	14	2.0	0.0005*	15	2.1	0.0005*	0.01	0.7	0.05
		female	37	0.7	13	1	4	0.8	0.6	11	0.6	0.2	9	0.7	0.2	0.6	0.4	0.5
RAPGEF1	internal plutonium	male	45	0.1	10	1	7	1.6	0.003	13	1.6	0.001*	15	1.4	0.04	0.009	0.4	0.004
		female	37	0.2	13	1	4	1.0	1.0	11	1.2	0.5	9	1.3	0.2	0.3	0.2	0.3
RNF166	internal plutonium	male	45	0.7	10	1	7	1.3	0.07	13	1.5	0.002	15	1.4	0.01	0.03	0.5	0.03
SERPINB9	internal plutonium	male	45	0.1	10	1	7	0.5	0.0007*	13	0.5	0.003	15	0.5	0.13	0.005	0.2	0.007
		female	36	0.4	12	1	4	0.5	0.4	11	0.5	1.0	9	0.5	0.3	0.7	0.6	1.0
SGSM2	internal plutonium	male	46	0.6	10	1	7	2.1	0.0008*	14	2.1	0.0004*	15	1.6	0.03	0.003	0.3	0.01
		female	37	0.6	13	1	4	1.5	0.1	11	1.4	0.3	9	1.5	0.1	0.4	0.9	0.8
SLC39A7	internal plutonium	male	46	0.8	10	1	7	1.4	0.009	14	1.5	0.0003*	15	1.28	0.04	0.007	0.4	0.007
		female	37	0.2	13	1	4	0.9	0.4	11	1.0	0.6	9	1.3	0.04	0.06	0.01	0.06
ZC3H7B	internal plutonium	female	28	0.048	9	1	3	1.4	0.2	10	1.6	0.1	6	2.0	0.008	0.04	0.02	0.2
		male	38	0.8	7	1	7	2.0	0.001	12	1.8	0.003	12	1.7	0.01	0.02	0.7	0.06
TRERF1	internal plutonium	female	37	0.0006*	13	1	4	1.2	0.5	11	1.5	0.2	9	2.1	0.01	0.03	0.03	0.01
		male	46	0.4	10	1	7	1.1	0.6	14	1.4	0.2	15	1.2	0.6	0.5	0.5	0.5
MGAT5	internal plutonium	male	40	0.06	7	1	7	1.8	0.01	12	1.8	0.005	14	1.6	0.05	0.04	0.6	0.02
	external γ ray	male	40	0.1	5	1	7	1.4	0.10	11	1.8	0.003	17	1.5	0.03	0.04	0.4	0.06
CLSTN3	internal plutonium	male	46	0.2	10	1	7	2.1	0.0006*	14	2.2	<0.0001*	15	2.0	0.001	0.002	0.6	0.009
		female	37	0.4	13	1	4	0.7	0.1	11	0.7	0.2	9	0.9	0.7	0.2	0.1	0.5
	external γ ray	male	46	0.08	7	1	7	1.7	0.01	14	1.3	0.1	18	1.6	0.01	0.047	0.3	0.06
		female	37	0.7	13	1	4	0.6	0.02	12	0.9	0.5	8	0.9	0.4	0.1	0.3	0.2
HNRNPA1	internal plutonium	male	46	0.09	10	1	7	1.6	0.049	14	1.7	0.01	15	1.1	0.5	0.01	0.04	0.003
	external γ ray	male	46	0.02	7	1	7	1.1	0.6	14	1.5	0.04	18	1.6	0.01	0.05	0.06	0.1
MCF2L	external γ ray	male	45	0.03	7	1	7	1.1	0.7	14	1.8	0.004	17	1.6	0.03	0.02	0.07	0.09
microRNA Transcriptome (n = 15)																		
miR-106b	internal plutonium	female	44	0.5	19	1	4	0.2	0.03	11	0.1	0.01	10	0.2	0.01	0.04	0.6	0.5
miR-185	internal plutonium	female	44	0.2	19	1	4	0.48	0.3	11	0.4	0.03	10	0.4	0.004	0.02	0.2	0.3
miR-484	internal plutonium	female	44	0.0005*	19	1	4	0.3	0.03	11	0.2	0.004	10	0.1	<0.0001*	<0.0001*	0.04	0.005
		male	48	0.6	12	1	7	0.7	0.2	14	1.1	0.7	15	1.3	0.3	0.07	0.05	0.4
miR-92a	internal plutonium	female	44	0.02	19	1	4	0.5	0.07	11	0.4	0.02	10	0.2	<0.0001*	0.0004*	0.06	0.01
		male	48	0.36	12	1	7	0.5	0.02	14	0.9	0.8	15	0.9	0.6	0.047	0.02	0.01
miR-423-5p	internal plutonium	female	43	0.01	19	1	4	0.5	0.2	11	0.3	0.03	9	0.2	0.0003*	0.002	0.03	0.04
		male	47	0.3	11	1	7	0.4	0.01	14	1.1	0.8	15	0.8	0.5	0.01	0.004	0.005
miR-451	internal plutonium	female	44	0.01	19	1	4	0.4	0.1	11	0.2	0.02	10	0.2	0.0004*	0.005	0.09	0.05
		male	48	0.1	12	1	7	0.3	0.01	14	1.0	1.0	15	1.0	0.9	0.01	0.006	0.02
miR-19b	internal plutonium	female	44	0.04	19	1	4	0.7	0.5	11	0.3	0.02	10	0.2	0.002	0.005	0.02	0.05
		male	48	0.2	12	1	7	0.4	0.008	14	0.9	0.8	15	0.9	0.9	0.01	0.004	0.03
miR-221	internal plutonium	male	47	0.04	11	1	7	0.3	0.003	14	0.8	0.6	15	0.6	0.09	0.009	0.02	0.003
miR-323-3p	internal plutonium	male	35	0.7	10	1	3	0.4	0.002	10	0.6	0.04	12	0.8	0.2	0.02	0.04	0.01

Continued on next page

TABLE 2
Continued.

			Dose linear		Dose categorical												3 df	2 df	KW 3 df
					Reference		I			II			III						
Gene symbol	Exposure	Strata	n	*P*	n	FC	n	FC	*P*	n	FC	*P*	n	FC	*P*		*P*	*P*	*P*
miR-151-3p	internal plutonium	female	43	0.01	18	1	4	15.0	0.05	11	13.1	0.08	10	48.3	0.002		0.009	0.4	0.08
	external γ ray	male	46	0.02	7	1	8	7.9	0.05	13	3.0	0.3	18	13.8	0.006		0.04	0.2	0.03
miR-342-5p	internal plutonium	female	42	0.9	18	1	4	0.3	0.003	11	0.3	0.005	9	0.4	0.01		0.04	0.3	0.3
	external γ ray	female	42	0.4	18	1	5	0.5	0.01	11	0.5	0.002	8	0.8	0.3		0.02	0.06	0.03
miR-501-5p	internal plutonium	female	44	0.3	19	1	4	0.7	0.3	11	0.3	0.006	10	0.3	0.002		0.008	0.05	0.004
	external γ ray	female	44	0.3	19	1	5	1	0.5	12	0.5	0.003	8	0.8	0.3		0.02	0.01	0.01
miR-18a	external γ ray	female	43	0.2	19	1	5	0.9	0.9	11	0.4	0.003	8	0.8	0.5		0.008	0.03	0.03
miR-339-3p	external γ	male	47	0.2	7	1	8	0.5	0.0002*	14	0.8	0.3	18	0.7	0.02		0.002	0.03	0.008
	ray	female	43	0.5	19	1	5	0.9	0.8	11	0.6	0.03	8	1.0	1.0		0.2	0.03	0.2
RNU48	internal	female	44	0.03	19	1	4	0.3	0.005	11	0.3	0.02	10	0.2	0.0002*		0.003	0.3	0.01
	plutonium	male	48	0.7	12	1	7	0.8	0.4	14	1.0	0.9	15	1.1	0.7		0.5	0.5	0.6

Notes. Normalized gene expression (normal distributed) served as the outcome variable in linear models. External gamma-ray exposure and internal plutonium burden were categorized and separately used as explanatory variables in linear and categorical models stratified by gender. Slope estimates were converted into fold changes (FC) in differential gene expression per dose category (inverse $\log_2$ transformed). *P* values were calculated per dose category, over all 4 groups (3 *df P* value) and among the 3 exposure groups include only adjustments for age at exposure (2 *df P* value). For significant 3 *df P* value dose-to-gene associations we also employed the nonparametric Kruskal-Wallis test and calculated a 3 *df P* value over all 4 groups. *P values ($P \leq 0.001$) that remain significant after Bonferroni correction for multiple comparisons. Associated gene names are in boldface. For these genes we show calculations regarding both genders to complete the picture. Absorbed dose ranges per dose category are (I) >0–0.055 Gy, (II) >0.055–0.085 Gy and (III) >0.085 Gy for internal alpha-particle exposure due to incorporated plutonium and (I) >0–0.5 Gy, (II) >0.5–1 Gy and (III) >1 Gy for external gamma-ray exposure.

than twofold gene expression differences in 95.2% and 90.5% downregulated genes, mostly in females (80%).

Correlation of Genes with Peripheral Blood Cell Counts

Among the 30 genes examined for associations with seven parameters of peripheral blood cell (PBC) counts (total of 210 comparisons) about 11% (24/210) appeared to be significantly associated with our gene expression values, but the gene expression variance explained by PBC counts did not exceed 10% in most (79%) of our models. Highest values for explained variance ranged between 15–20% and were observed in about 1% (2/210) of our models.

Examinations on MicroRNA –mRNA Target Relationships

Five microRNAs (miR-92a, miR-484, miR-106b, miR-323-3p and miR-19b) that targeted nine mRNAs are depicted in Fig. 3. Our GTPase associated genes (Fig. 3, indicated with an asterisk), which showed a binary (on/off) dose-to-gene relationship in males (Table 1) were also targeted by microRNAs with significant dose-to-gene relationships in females (miR-484, miR-106b), so that we do not completely understand their microRNA control. For the other microRNAs we found partially dose-to-gene associations corresponding to their target genes (linear dose-to-gene relationship in females for miR-19b with TRERF1 or miR-484 with ZC3H7B). However, this was not consistently seen in all of the microRNA-mRNA target relationships that were examined. For instance, ''other'' dose-to-gene relationships in males were observed for miR-19b and miR-323-3p with binary gene dose relationships of their target genes, namely CLSTN3 and HNRNPA1 (Fig. 3).

DISCUSSION

In previous studies we screened the entire mRNA transcriptome and large parts of the microRNA transcriptome (667 microRNAs) to search for dose-to-gene relationships in 40 healthy, former nuclear weapon production workers exposed to internal plutonium and/or external gamma rays to find out whether these relationships might persist decades after exposure. We identified 140 candidate genes (95 mRNA and 45 microRNA species) and examined them in the context of the study shown herein. Dose-to-gene relationships of these 140 genes were validated employing qRT-PCR in an independent group consisting of another 92 healthy, former nuclear weapon production workers. We found significant dose-to-gene associations in 15 mRNA and 15 microRNAs with

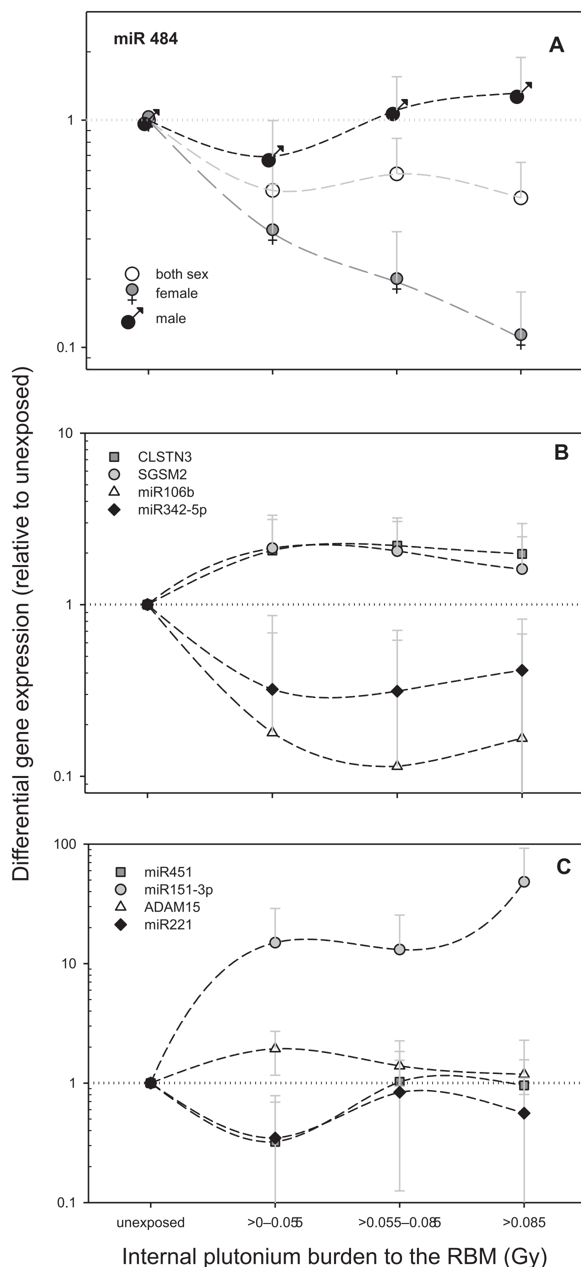

FIG. 2. Differential gene expression (relative to the unexposed group arbitrarily set to 1) was examined over three exposure groups with absorbed dose (Gy) from internal alpha-particle exposure due to incorporated plutonium to the red bone marrow (RBM). Dose-to-gene associations appeared gender dependent and followed three patterns, namely linear, binary and other relationships. Appropriate genes were selected and are visualized in this figure. Panel A reflects the sex dependency of gene expression data making stratification necessary. After stratification a significant linear dose-to-gene relationship for females only becomes apparent. Panel B describes so-called binary or on/off dose-to-gene relationships characterized by almost constant gene expression changes over all dose categories. Two examples are shown for upregulated (CLSTN3 and SGSM2) and downregulated genes (miR106b and miR342-5p). Panel C describes dose-to-gene relationships that do not follow a linear or binary pattern, but are characterized by significant changes in gene expression taking place in only one dose category or show shapes other than linear or binary. Error bars refer to the 95% confidence interval and are sometimes shown only in one direction for clarification purposes. To better visualize exposure-to-gene associations we connected data points with a spline curve.

similarities (predominantly associations with internal plutonium body burden, but of linear type in 20% only) and differences (gender, fold change and direction of gene regulation).

In agreement with our previous whole genome analysis study (*9*) we again found persistent dose-to-gene associations in favor of the incorporated plutonium, but using a different technique for gene expression measurements (qRT-PCR) and another group of exposed individuals. From the biological viewpoint, we expected these results based on the constant alpha-ray exposure due to incorporated plutonium. The chronic external gamma-ray exposure of our group lasted over two decades leading to less but still significant dose-to-gene associations, which were detectable, even up to three decades after chronic radiation exposure. Such findings are in agreement with previous work on Chernobyl thyroid cancer patients after iodine-131 exposure (*7, 8, 22*) and support the concept that radiation exposure somehow changes the default of the microRNA transcriptome that can still be detected in cells originating from directly exposed cells many cell generations afterwards. Whether these gene expression changes might be caused by persistent intrachromosomal aberrations (*3, 4*), complex aberrations (*2*), gain of chromosome bands (*23*), or distinct pattern of oncogene rearrangements (*24*), copy number alterations (*25*) or epigenetic modifications (*26, 27*) as suggested, for example, in the context of iodine-131 exposures of Chernobyl accident victims (*7*) remains to be shown in future studies.

Examinations of the mRNA transcriptome in our study showed primarily a binary dose-to-gene relationship with genes being similarly downregulated over all three dose categories relative to the unexposed controls (on/off pattern). Comparable dose-to-gene relationships were not cited, but at least six (partly closely related genes) from our 15 mRNAs had already been reported to be associated with iodine-131 exposure based on microarray measurements in the normal thyroid tissues of Chernobyl victims (Supplementary Table S1; http://dx.doi.org/10.1667/RR13645.1.S1). Of the microRNA transcriptome we found reports for 5 of our 15 microRNAs to be associated with radiation exposure (Supplementary Table S1; http://dx.doi.org/10.1667/RR13645.1.S1). Hence, chronic protracted radiation exposures (absorbed dose) to either internal alpha particles due to incorporated plutonium (0.003–1.01 Gy) or external gamma rays (0.4–3.1 Gy) to the red bone marrow appears to induced a similar biological response. Whether these gene expression changes might be associated with an increased health risk of a chronic non-cancer disease such as cardiovascular diseases needs to be examined in future work.

In general, our investigations showed that while most of the microRNAs were downregulated, the majority of the mRNAs that they targeted were upregulated, which is what would typically be expected from a biological point of view. However, one must bear in mind that predicting the direction of mRNA gene expression controlled by several

TABLE 3
A Summary of the Characteristics of Dose-to-Gene Relationships Presented in Table 2

	mRNA transcriptome		microRNA transcriptome		Fisher's exact test
	n	Percentage	n	Percentage	*P* value
Gender (per gene, n = 15/15)					
female	1	6.7	8	53.3	
male	13	86.7	3	20.0	
both	1	6.7	4	26.7	0.004
Exposures (per gene, n = 15/15)					
internal plutonium	11	73.3	10	66.7	
both exposures	3	20.0	3	20.0	
external γ ray	1	6.7	2	13.3	0.9
Gene-dose relationship (n = 19/21)					
linear[1]	4	21.1	4	19.0	
binary[2]	14	73.7	8	38.1	
other[3]	1	5.3	9	42.9	0.009
Differential gene expression (n = 19/21)[4]					
upregulated	18	94.7	2	9.5	
downregulated	1	5.3	19	90.5	5.3×10^{-7}
Fold difference (n = 19/21)[5]					
$\leq$2-fold	17	89.5	1	4.8	
$\geq$2–5-fold	2	10.5	15	71.4	
>5-fold	0	0.0	5	23.8	4.3×10^{-7}

Notes. A summary of the characteristics of dose-to-gene relationships presented in Table 2 are shown here, statistically examined for frequency differences for 15 mRNAs and 15 microRNAs and 19/21 dose-to-gene associations, respectively. The characteristics of dose-to-gene relationships comprise the dependency on gender and exposure type as well as the shape, direction (up/downregulated) and height (fold difference) of the dose-to-gene relationship.

[1] Linear association: significant *P* value for linear association and at least borderline significant 2 *df P* value.

[2] Binary: significant 3 *df P* value and associations in the same direction and fold-change difference below 30% over all dose categories.

[3] Others: remainder dose-to-gene relationships.

[4] Differential gene expression is defined as up/downregulated provided the fold-difference appears >1.5/<0.67 in at least one dose category.

[5] Fold-difference categories are shown when identified in at least one dose category.

microRNA [networks (Fig. 3)] is challenging. In our study, dose-to-gene associations were complex with about half (43%) of our microRNAs showing dose-to-gene relationships other than binary or linear. It is unclear how these differences in dose-to-gene response might be connected, but they might provide evidence for the interplay of different microRNAs that nonetheless result in a binary response of their mRNA target genes (Fig. 3). Interestingly, at the microRNA transcriptome associations with females predominated in opposition to male associations at the mRNA transcriptome (Table 2). This pattern should be judged cautiously due to the almost inverse gender relationship, with about 73% females in the unexposed group and 61–88% males in the exposed groups, which certainly complicates interpretations. At the microRNA transcriptome level we detected a number of female dependent dose-to-gene relationships that appeared to be neither linear nor binary and showed higher fold difference in gene expression compared to the mRNA transcriptome. Whether or not these differences are linked to gender remains unclear.

Examinations of microRNA–mRNA target relationships showed that linear dose-to-gene relationships of microRNAs were translated into linear dose-to-gene relationships for targeted mRNAs, but this was not universally observed especially in nonlinear dose-to-gene relationships and probably due to the low number of target-relationships inherent in our study, and therefore caution should be taken in making interpretations in this direction (Fig. 3).

Four of our 15 mRNAs represented genes associated to GTPases. All of these showed a binary dose-to-gene relationship and were downregulated. GTPases are known to act as an on/off switch for the regulation of different signal transduction cascades (*28, 29*). It could be hypothesized that functionality and biological control are linked in this way. Interestingly, gene enrichment analysis in normal thyroid tissue of Chernobyl victims exposed to iodine-131 indicated an underrepresented G-protein (GTPase) coupled receptor, thus corroborating our findings. Alternatively, it might be possible that for many of our examined genes the differences in chronic radiation exposure over our three dose categories simply will not be translated into different biological responses.

The remaining genes of our 15 mRNAs are associated with changes in cell adhesion (ADAM15, MGAT5, CLSTN3), mRNA modulation and transport (HNRNPA1, TRERF1, ATF6B), defense mechanism (SERPINB9) or missed detailed annotations [SLC39A7, ZC3H7B, LOC731275, RNF166 (Supplementary Table S1; http://dx.doi.org/10.1667/RR13645.1.S1)]. As previously reported, biological processes such as mRNA modulation and transport appear to be significantly associated with iodine-

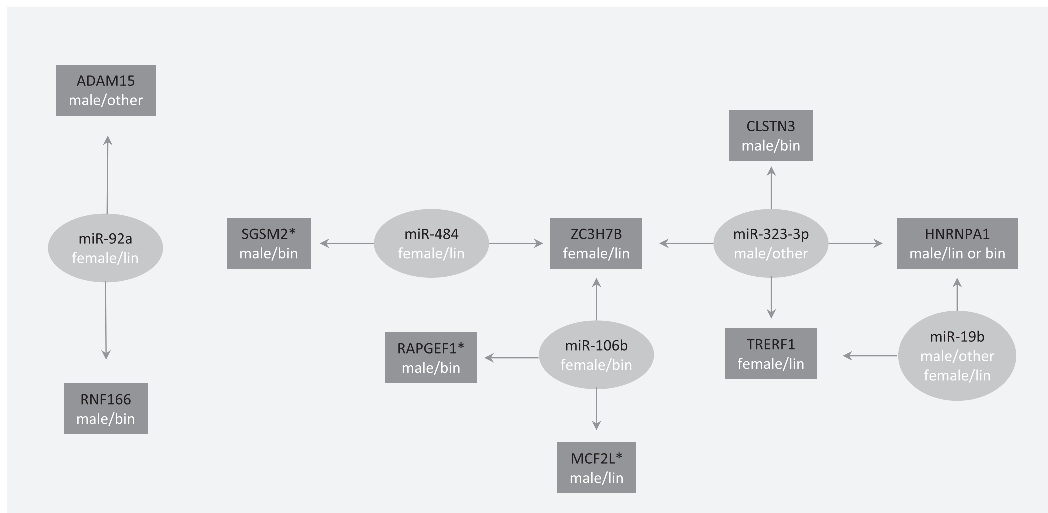

FIG. 3. Target scans of microRNA-mRNA target-relationships. Arrows indicate differentially expressed microRNA (ellipse)–mRNA (squares) target-relationship. For instance, genes were targeted by microRNAs as follows: MCF2L by 1 microRNA (miR-106b) and ZC3H7B by 3 microRNAs. GTPase associated genes are marked with an asterisk. Significant linear (/lin), binary (on/off, /bin) and others (/other) dose-to-gene relationships are shown for males and females using white letters.

131 exposure in thyroid tissue of Chernobyl victims employing gene enrichment analysis (*8*). Known annotations of our 15 microRNAs have been associated with proliferation and migration as well as with diseases have been reported (Supplementary Table S1; http://dx.doi.org/10.1667/RR13645.1.S1). After identifying genes associated with radiation exposure in this study, we now plan to examine the potential associations of these genes with certain non-cancer diseases in further studies.

We initially wondered about the strong gender dependent expression of mRNAs as well as microRNAs, however, the gender-specific radiation-induced changes of the microRNA transcriptome have already been reported in different brain regions of a mouse model (*30*). However, there are several other limitations to keep in mind when interpreting the results of our study. With 150 individuals examined at the mRNA transcriptome and for the first time at the microRNA transcriptome as well, our study represents one of the largest and most complete gene expression *in vivo* studies based on radiation exposed cohorts. However, despite this our numbers are still relatively low and become even smaller after stratification by gender, thus reducing the power of our analyses. We also have to take into account multiple confounders such as chronic diseases, because gene expression was measured in the peripheral blood reflecting changes taking place in different parts of the whole body. Even though our database appeared to be very detailed, we cannot be completely certain that we have adjusted for all potential confounders and even residual confounding is an issue when considering the more general available information for some of our variables such as alcohol consumption or smoking (yes/no). Furthermore, we did not have organ dose estimates from medical diagnostic procedures for our controls. However, in our occupationally exposed individuals medical diagnostic exposures to the RBM were always $<1\%$ of the occupational external gamma-ray exposure, thus making false associations unlikely. We also examined whether gene expression changes of our genes originated from blood cell counts of the whole blood. However, significant associations among gene expression and blood cell counts were found in only 10% and in these models about 90% of gene expression variance could not be explained by blood cell counts. Therefore, gene expression changes might be primarily caused by measuring circulating mRNA/microRNA originating from the serum or circulating cells. Because of the explorative nature of our study we did not use *P* values adjusted for multiple comparisons as a selection criteria. However, 15 mRNAs and 15 miRNAs showed significant exposure-to-gene associations in both stages of our study and remained eligible using two different methods (microarrays vs. qRT-PCR) and examining two different groups (40 samples in phase I and 92 samples in phase II). In addition, eight of our mRNAs and six of our miRNAs passed the Bonferroni correction applied to these selected genes.

In summary, we found exposure and gender-specific changes at the mRNA- and microRNA transcriptome persisting after occupational chronic radiation exposures, which supports the concept that permanent post-exposure signatures might exist.

SUPPLEMENTARY INFORMATION

Supplementary Table S1. Annotation of our 15 mRNA and 15 microRNA candidate genes.

ACKNOWLEDGMENTS

This work was supported by the German Ministry of Defense. We appreciate the technical assistance provided by Mrs. Taubert in the isolation of total RNA.

Received: December 19, 2013; accepted: May 27, 2014; published online: July 30, 2014

REFERENCES

1. United Nations Scientific Committee on the Effects of Atomic Radiation. UNSCEAR 2000 report to the General Assembly, with scientific annexes. Sources and effects of ionizing radiation. United Nations, New York: UNSCEAR; 2000.
2. Hande MP, Azizova TV, Burak LE, Khokhryakov VF, Geard CR, Brenner DJ. Complex chromosome aberrations persist in individuals many years after occupational exposure to densely ionizing radiation: an mFISH study. Genes Chromosomes Cancer 2005; 44:1–9.
3. Hande MP, Azizova TV, Geard CR, Burak LE, Mitchell CR, Khokhryakov VF. Past exposure to densely ionizing radiation leaves a unique permanent signature in the genome. Am J Hum Genet 2003; 72:1162–70.
4. Mitchell CR, Azizova TV, Hande MP, Burak LE, Tsakok JM, Khokhryakov VF, et al. Stable intra-chromosomal biomarkers of past exposure to densely ionizing radiation in several chromosomes of exposed individuals. Radiat Res 2004; 162:257–63.
5. Anspaugh LR, Degteva MO, Vasilenko EK. Mayak. Production association: introduction. Radiat Environ Biophys 2002; 41:19–22.
6. Azizova TV, Day RD, Wald N, Muirhead CR, O'Hagan JA, Sumina MV, et al. The ''clinic'' medical-dosimetric database of Mayak production association workers: structure, characteristics and prospects of utilization. Health Phys 2008; 94:449–58.
7. Abend M, Pfeiffer RM, Ruf C, Hatch M, Bogdanova TI, Tronko MD, et al. Iodine-131 dose dependent gene expression in thyroid cancers and corresponding normal tissues following the Chernobyl accident. PLoS One 2012; 7:e39103.
8. Abend M, Pfeiffer RM, Ruf C, Hatch M, Bogdanova TI, Tronko MD, et al. Iodine-131 dose-dependent gene expression: alterations in both normal and tumor thyroid tissues of post-Chernobyl thyroid cancers. Br J Cancer 2013; 109:2286–94.
9. Abend M, Azizova T, Müller K, Dörr H, Senf S, Kreppel H, et al. Gene expression analysis in Mayak workers with occupational prolonged radiation exposure. Health Phys 2014; 106:664–76.
10. Khokhryakov VF, Kudravtseva TI, Chernikov VI, Suslova KG, Orlova IA, Filipy RE. A scintillation method for determination of actinide alpha-activity in samples. J Radioanal Nucl Chem 1998; 234:293–5.
11. Krahenbuhl MP, Slaughter DM, Wilde JL, Bess JD, Miller SC, Khokhryakov VF, et al. The historical and current application of the FIB-1 model to assess organ dose from plutonium intakes in Mayak workers. Health Phys 2002; 82:445–54.
12. Fountos BN, Rabovsky JL. The Department of Energy's Russian Health Studies Program. Health Phys 2007; 93:187–9.
13. Vasilenko EK, Scherpelz RI, Gorelov MV, Strom DJ, Smetanin MYu. External dosimetry reconstruction for Mayak workers. AAHP Special Session Health Physics Society Annual Meeting 2010. (http://www.hps1.org/aahp/public/AAHP_Special_Sessions/2010_Salt_Lake_City/pm-1.pdf)
14. Vasilenko EK, Khokhryakov VF, Miller SC, Fix JJ, Eckerman K, Choe DO, et al. Mayak worker dosimetry study: an overview. Health Phys 2007; 93:190–206.
15. Khokhryakov VF, Menshikh ZS, Suslova KG, Kudryavtseva TI, Tokarskaya Z, Romanov SA. Plutonium excretion model for the healthy man. Radiat Protec Dosim 1994; 53:235–9.
16. Khokhryakov VV, Khokhryakov VF, Suslova KG, Vostrotin VV, Vvedensky VE, Sokolova AB, et al. Mayak Worker Dosimetry System 2008 (MWDS-2008): assessment of internal dose from measurement results of plutonium activity in urine. Health Phys 2013; 104:366–78.
17. Khokhryakov VF, Suslova KG, Vostrotin VV, Romanov SA, Menshikh ZS, Kudryavtseva TI, et al. The development of the plutonium lung clearance model for exposure estimation of the Mayak Production Association, nuclear plant workers. Health Phys 2002; 82:425–31.
18. Khokhryakov V, Suslova K, Aladova E, Vasilenko E, Miller SC, Slaughter DM, et al. Development of an improved dosimetry system for the workers at the Mayak Production Association. Health Phys 2000; 79:72–6.
19. Romanov SA, Vasilenko EK, Khokhryakov VF, Jacob P. Studies on the Mayak nuclear workers: dosimetry. Radiat Environ Biophys 2002; 41:23–28.
20. Romanyukha AA, Ignatiev EA, Vasilenko EK, Drozhko EG, Wieser A, Jacob P, et al. EPR dose reconstruction for Russian nuclear workers. Health Phys 2000; 78:15–20.
21. Hall E, Giaccia AJ. Radiobiology for the radiologist, 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2012.
22. Dom G, Tarabichi M, Unger K, Thomas G, Oczko-Wojciechowska M, Bogdanova T, et al. A gene expression signature distinguishes normal tissues of sporadic and radiation-induced papillary thyroid carcinomas. Br J Cancer 2012; 107:994–1000.
23. Hess J, Thomas G, Braselmann H, Bauer V, Bogdanova T, Wienberg J, et al. Gain of chromosome band 7q11 in papillary thyroid carcinomas of young patients is associated with exposure to low-dose irradiation. Proc Natl Acad Sci 2011; 108:9595–600.
24. Nikiforov YE, Rowland JM, Bove KE, Monforte-Munoz H, Fagin JA. Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. Cancer Res 1997; 57:1690–4.
25. Stein L, Rothschild J, Luce J, Cowell JK, Thomas G, Bogdanova TI, et al. Copy number and gene expression alterations in radiation-induced papillary thyroid carcinoma from Chernobyl pediatric patients. Thyroid 2010; 20:475–87.
26. Aypar U, Morgan WF, Baulch JE. Radiation-induced genomic instability: are epigenetic mechanisms the missing link? Int J Radiat Biol 2011; 87:179–91.
27. Herceg Z, Vaissiere T. Epigenetic mechanisms and cancer: an interface between the environment and the genome. Epigenetics 2011; 6:804–19.
28. Neuwald AF. The charge-dipole pocket: a defining feature of signaling pathway GTPase on/off switches. J Mol Biol 2009; 390:142–53.
29. Dohlman HG, Thorner J. RGS proteins and signaling by heterotrimeric G proteins. J Biol Chem 1997; 272:3871–4.
30. Koturbash I, Zemp F, Kolb B, Kovalchuk O. Sex-specific radiation-induced microRNAome responses in the hippocampus, cerebellum and frontal cortex in a mouse model. Mutat Res 2011; 722:114–8.