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SUMMARY

This project sought mechanistic understanding of the epigenetic response of tissues as well as the consequences of those responses, when induced by low dose irradiation in a well-established model system (mouse). Based on solid and extensive preliminary data we investigated the molecular epigenetic mechanisms of *in vivo* radiation responses, particularly – effects of low, occupationally relevant radiation exposures on the genome stability and adaptive response in mammalian tissues and organisms. We accumulated evidence that low dose irradiation altered epigenetic profiles and impacted radiation target organs of the exposed animals.

The main long-term goal was to dissect the epigenetic basis of induction of the low dose radiation-induced genome instability and adaptive response and the specific fundamental roles of epigenetic changes (i.e. DNA methylation, histone modifications and miRNAs) in their generation.

We hypothesized that changes in global and regional DNA methylation, global histone modifications and regulatory microRNAs played pivotal roles in the generation and maintenance low-dose radiation-induced genome instability and adaptive response. **We predicted** that epigenetic changes influenced the levels of genetic rearrangements (transposone reactivation). **We hypothesized** that epigenetic responses from low dose irradiation were dependent on exposure regimes, and would be greatest when organisms are exposed in a protracted/fractionated manner: fractionated exposures > acute exposures. **We anticipated** that the epigenetic responses were correlated with the gene expression levels.

Our immediate objectives were:

- To investigate the exact nature of the global and locus-specific DNA methylation changes in the LDR exposed cells and tissues and dissect their roles in adaptive response
- To investigate the roles of histone modifications in the low dose radiation effects and adaptive response
- To dissect the roles of regulatory microRNAs and their targets in low dose radiation effects and adaptive response
- To correlate the levels of epigenetic changes with genetic rearrangement levels and gene expression patterns.

In sum, we determined the precise global and locus-specific DNA methylation patterns in the LDR-exposed cells and tissues of mice, and to correlated DNA methylation changes with the gene expression patterns and manifestations of genome instability. **We also** determined the alterations of global histone modification pattern in the LDR exposed tissues. **Additionally, we established** the nature of microRNAome changes in the LDR exposed tissue.

In this study we for the first time found that LDR exposure caused profound tissue-specific epigenetic changes in the exposed tissues. We established that LDR exposure affect methylation of repetitive elements in the murine genome, causes changes in histone methylation, acetylation and phosphorylation. Importantly, we found that LDR causes profound and persistent effects on small RNA profiles and gene expression, and that miRNAs are excellent biomarkers of LDR exposure.

Furthermore, we extended our analysis and studied LDR effects in rat tissues and human tissues and cell lines. There we also analyzed LDR-induced gene expression, DNA methylation and miRNA changes. Our datasets laid foundation for several new research projects aimed to

understand molecular underpinnings of low dose radiation responses, and biological repercussions of low dose radiation effects and radiation carcinogenesis.

SOME KEY RESULTS

ESTABLISHMENT OF THE TISSUE BANK - ANIMAL EXPOSURE AND TISSUE PROCUREMENT

1) Animal exposures:

The following 6 animals groups were set up:
Group 1 - sham-treated cohort;
Group 2 - exposed cohort – acute dose of 0.01 Gy;
Group 3 - exposed cohort – acute dose of 0.1 Gy;
Group 4 - exposed cohort – acute dose of 1 Gy;
Group 5 - exposed cohort – fractionated dose – 10 x 0.1 Gy;
Group 6 - primed and exposed cohort – 0.1 Gy followed by 1 Gy.
Each group of animals consisted of 18 sexually mature (50 days old) C57Bl/6 male mice (6 animals per time point). All animals were humanely sacrificed 6 hours, 96 hours or 4 weeks after completion of the treatment protocol. The 6 hours time-point is used to analyze the initial changes, while the 96 hours and 4 weeks time-points will help to delineate the long-term persistent changes.
We harvested radiation target tissues (spleen and thymus). Additionally, we harvested liver, gonads, skin and brain tissues. Experiments were independently repeated twice.

2) In parallel, we analyzed effects of low (0.05, intermediate -0.5, and high – 5 Gy) of radiation in a murine model.

Radiation exposure: fifty-day-old mice (15 males) were randomly assigned to different treatment groups. Five males received 0.05 Gy of X-ray exposure to the entire body (90 kV, 5 mA). Five animals received 5 Gy of X-ray exposure to the entire body (90 kV, 5 mA). Five were sham treated and served as control. Animals were sacrificed 24 hours after exposure. Spleens were harvested immediately following sacrifice.

3) Additionally, the following groups were set up to further analyze priming and adaptive effects:

A control group: sham-treated controls (0/0); a low-dose group (0.3/0): mice were exposed to only 0.3 Gy irradiation on day 0; a high-dose group (0/3): mice were exposed to only 3 Gy acute irradiation on day 4; a priming group (0.3/3): mice were exposed to both 0.3 Gy priming irradiation on day 0 and 3 Gy acute irradiation on day 4. Twenty mice per group (10 males and 10 females) were sacrificed at 6 hours, 96 hours, and two weeks after irradiation.

4) Cell line exposures:

Various cells lines (MCF-7 breast adenocarcinoma cells, normal mammary gland epithelial cells (HuMEC), WI-38 fibroblasts, glioblastoma cell lines) were exposed to several low, intermediate and high doses of radiation and harvested at different time points after exposure. We also analyzed LDR responses as a function of cellular aging by comparing effects in young and senescent cells.

KEY EXPERIMENTAL RESULTS

PART I
LDR-INDUCED CHANGES IN MURINE MODEL
LIVER TISSUE

LDR exposure affected gene expression in murine liver. Table below shows a total number of regulated liver transcripts in low-dose X-irradiated C57BL/6 mice

Table 1. Total number of regulated liver transcripts in low-dose X-irradiated C57BL/6 mice, threshold cut-off 0.59 in both directions

	Total number of regulated transcripts	1 Gy			0.1 Gy			0.01 Gy		
6 hrs	999	16	↑11	69%	7	↑4	57%	521	↑494	95%
			↓5	31%		↓3	43%		↓27	5%
96 hrs	655	5	↑1	20%	69	↑54	78%	88	↑62	70%
			↓4	80%		↓15	22%		↓26	30%
4 wks	596	7	↑1	14%	320	↑7	2%	159	↑8	5%
			↓6	86%		↓313	98%		↓151	95%

Data on changes in gene expression after X-irradiation. The total number of regulated transcripts in liver tissue is given with the number of up- (arrows pointing up) and down-regulated (arrows pointing down) transcripts given as the total number and percentage.

Most significantlyderegulated genes are presented below:

Table 2. Strongest modulated transcripts per acute exposure

	6 hrs		96 hrs		4 wks	
	Up	**Down**	**Up**	**Down**	**Up**	**Down**
1 Gy	*Cyp7a1* (1.99) *Mfsd2* (1.44) *Gadd45g* (1.20) *Ccrn4l* (1.10) *Tsc22d3* (1.08) *Ddit4* (1.06) *Gm129* (1.02) *Ppp1r3c* (0.84) *Rgs16* (0.83) *Spata2L* (0.80)	*Osgin1* (-1.06) *Arrdc3* (-0.90) *Hspa8* (-0.86) *Slc25a30* (-0.79) *Akr1c12* (-0.66)	*Cyp4a14* (1.45)	*Car3* (-1.02) *Bcl6* (-0.86) *Hhex* (-0.66) *Hspa8* (-0.60)	*Mt1* (1.14)	*8430408G22Rik* (-0.89) *LOC677317* (-0.86) *Stard4* (-0.84) *LOC100048105* (-0.80) *Srr* (-0.79) *Mrpl27* (-0.69)
0.1 Gy	*Gadd45g* (1.90) *Cyp7a1* (1.47) *Prodh* (1.38) *Gm129* (1.02)	*St6gal1* (-0.69) *Litaf* (-0.67) *Ssr3* (-0.66)	*Arrdc3* (1.60) *LOC100047935* (1.58) *LOC100048187* (1.18) *Ndufb9* (1.14) *Fgg* (1.14) *LOC100048480* (1.11) *Dbp* (1.08) *Aamp* (1.04) *Cox6c* (1.03) *Pgm2* (0.92)	*Ephx1* (-1.12) *Bcl6* (-1.10) *Sgk1* (-1.05) *Ide* (-0.88) *Cml4* (-0.75) *Atp5c1* (-0.71) *Pter* (-0.68) *Acsl5* (-0.66) *Mapk14* (-0.66) *Cct6a* (-0.65)	*Mt1* (0.93) *H2-Ab1* (0.84) *Decr2* (0.73) *C1qb* (0.71) *Rps2* (0.69) *H2-DMa* (0.64) *Cfp* (0.63)	*Fgl1* (-1.72) *Mettl7b* (-1.70) *Krt8* (-1.69) *LOC100048480* (-1.68) *LOC100039532* (-1.64) *Cfb* (-1.59) *Cyb5* (-1.59) *Slc35b1* (-1.58) *Pigr* (-1.52) *Pgrmc1* (-1.52)
0.01 Gy	*Cfb* (2.56) *LOC100047762* (2.34) *Mettl7b* (2.27) *Fgl1* (2.13) *Gadd45g* (2.13) *Slc38a4* (2.07) *H2-Q8* (2.03) *Ndufb9* (2.02) *Slc25a25* (1.84) *Apcs* (1.81)	*Clec4g* (-1.29) *Hba-a1* (-1.09) *Aqp1* (-1.07) *F2r* (-0.96) *Ly6a* (-0.89) *Osgin1* (-0.88) *Dct* (-0.87) *Bik* (-0.85) *Lgals3* (-0.84) *Eltd1* (-0.80)	*Arrdc3* (1.78) *LOC100047935* (1.70) *Rpl36a* (1.35) *Fgg* (1.27) *Ndufa6* (1.04) *Aamp* (1.04) *LOC100048187* (1.03) *LOC100048480* (0.98) *1110001J03Rik* (0.97) *Cfb* (0.97)	*Aacs* (-1.10) *Mvd* (-0.90) *Ide* (-0.89) *Fcna* (-0.89) *Hspa8* (-0.84) *Cyp4a12a* (-0.74) *Rps11* (-0.72) *EG668668* (-0.70) *Rps4x* (-0.70) *Acsl5* (-0.70)	*Ugt1a10* (0.71) *Aqp9* (0.68) *Aldh3a2* (0.66) *Clptm1l* (0.65) *Clec4g* (0.63) *1810055G02Rik* (0.62) *St3gal5* (0.61) *Hsd17b11* (0.60)	*Slc35b1* (-1.51) *Slc25a25* (-1.49) *LOC100048480* (-1.40) *Cfb* (-1.38) *Rps21* (-1.35) *Krt8* (-1.29) *LOC100039532* (-1.28) *LOC100047935* (-1.27) *Fgl1* (-1.27) *Pigr* (-1.24)

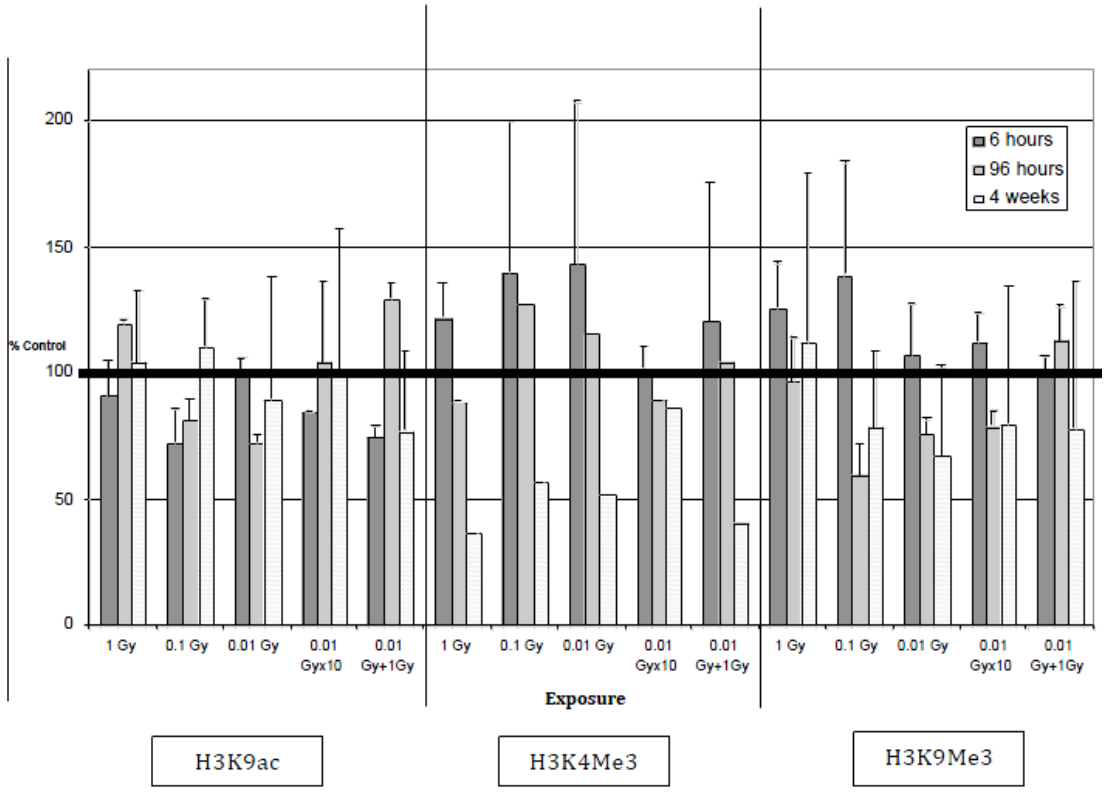
Up to ten of the most strongly up- and down-regulated transcripts in liver. Number in parentheses indicate fold change.

Several genes were common between all groups:

Table 3. Common modulated transcripts between exposure groups

	Acute doses (1 Gy, 0.1 Gy, 0.01 Gy)		
	1 Gy	**0.1 Gy**	**0.01 Gy**
6 hrs	2 total; ↑2		
	Cyp7a1 (1.99) *Gadd45g* (1.20)	*Cyp7a1* (1.47) *Gadd45g* (1.90)	*Cyp7a1* (1.66) *Gadd45g* (2.13)
96 hrs	None		
	-	-	-
4 wks	3 total; ↓3		
	LOC677317 (-0.89) *Mrpl27* (-0.79) *Stard4* (-0.69)	*LOC677317* (-0.92) *Mrpl27* (-0.84) *Stard4* (-1.02)	*LOC677317* (-0.86) *Mrpl27* (-0.69) *Stard4* (-0.84)

We also noted changes in levels of global histone modifications.

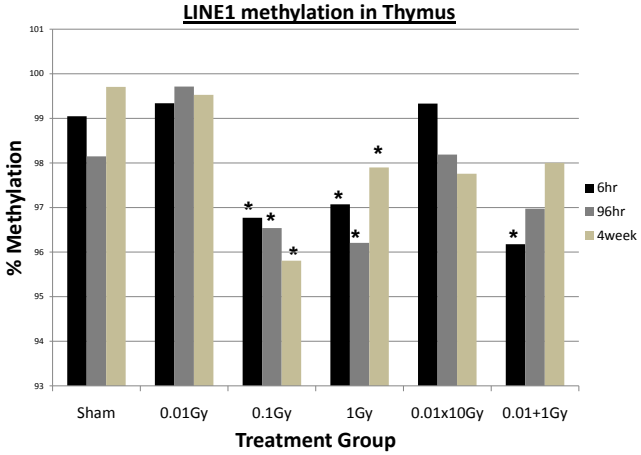


Additionally, we found alterations in level of DNA methylation, cell cycle and apoptosis-associated proteins.

THYMUS TISSUE

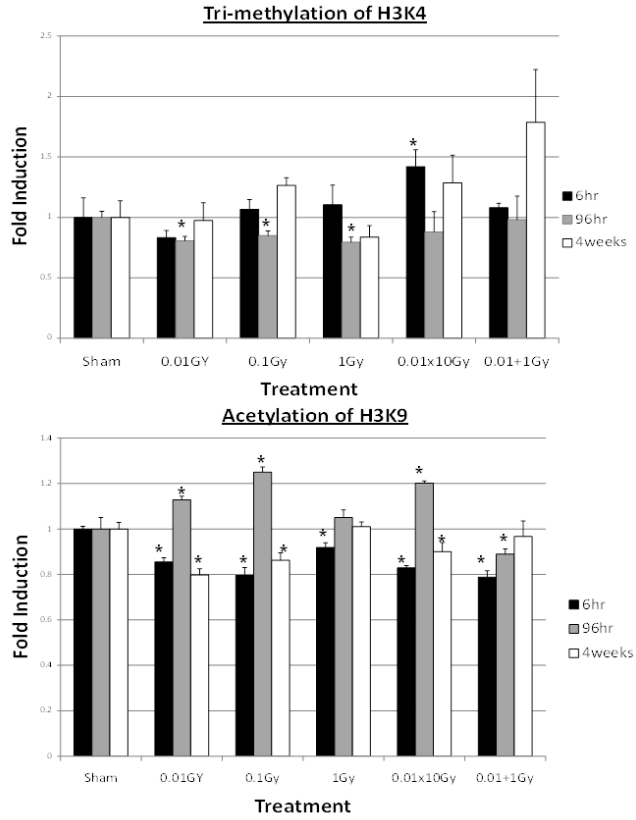
We analyzed the levels of LINE1 DNA methylation in thymus of LDR-exposed mice. We found significant changes in the thymus at 1.0 and 0.1 Gy exposure at all time-points and in the adaptive response cohort at 6hrs only. Increase of LINE1 methylation in the priming/adaptive cohort may suggest priming effects indeed exist and may be protective in nature.

Figure 1– LINE11 methylation in thymic tissues of exposed animals



We also observed changes in global histone modifications.

Figure 2 – levels of histone modification changes in thymic tissues of exposed mice



LDR exposure profoundly affected miRNA levels in murine thymus. MiRNAs are excellent biomarkers of LDR exposure. Moreover, in thymus LDR exposure caused a much more profound deregulation of miRNAs than 1 Gy exposure.

Low dose radiation-induced epigenetic changes in an animal model: microRNA analysis

Objective: To determine the nature of microRNAome changes in the LDR exposed tissue.

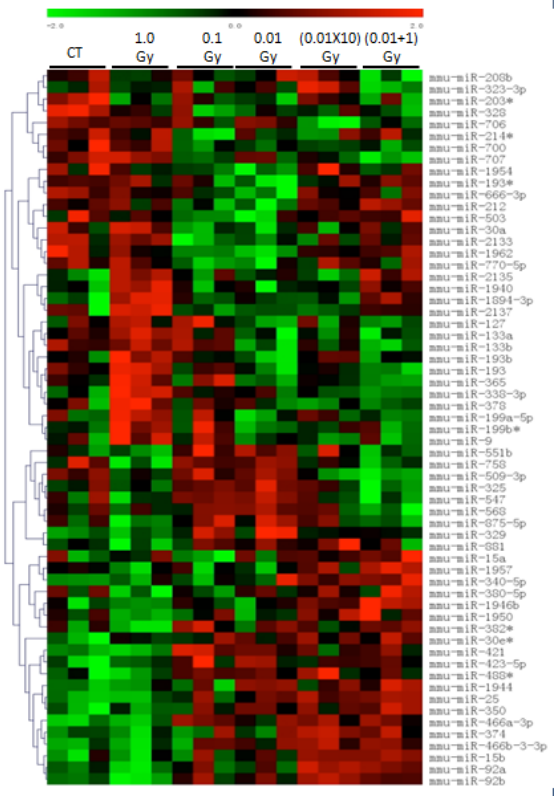
Approach: Emerging evidence indicates that small regulatory RNAs (microRNAs) are important regulators of many cellular functions including proliferation, differentiation, and cell death. At specific time intervals we determined microRNA expression profiles of control and LDR exposed tissues.

Results:

-LDR exposure led to significant and persistent microRNAome changes in the exposed tissues.

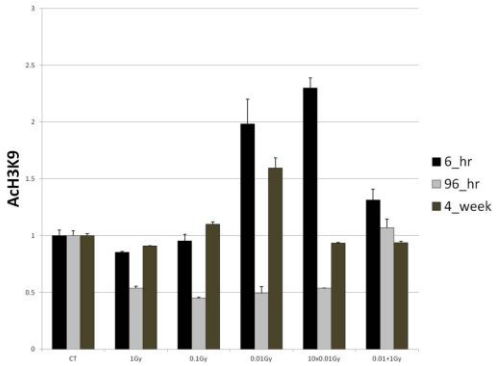
-Altered small RNAs affect expression of numerous target proteins that control processes such as apoptosis, cell cycle control, DNA repair, inflammation, signalling.

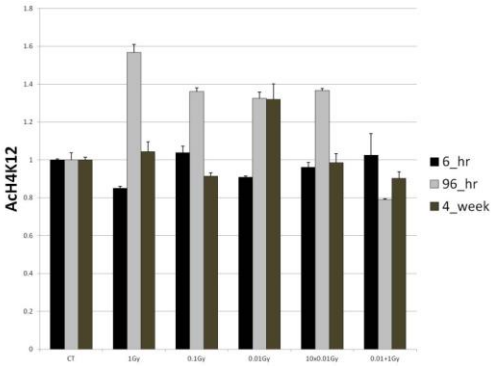
-miRNA expression patterns are BETTER LDR biomakers than gene expression patterns!!!

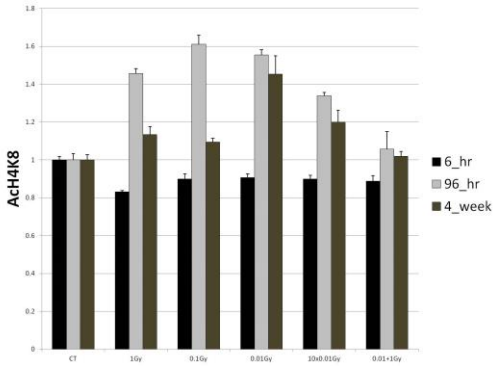


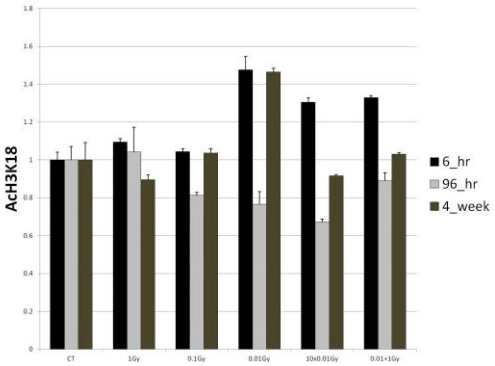
SPLEEN TISSUE

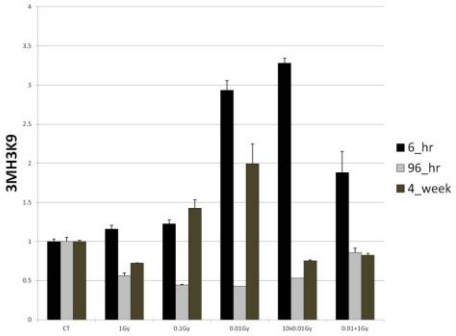
LDR exposure significantly affected histone methylation profiles in murine spleen, as seen in figures below.

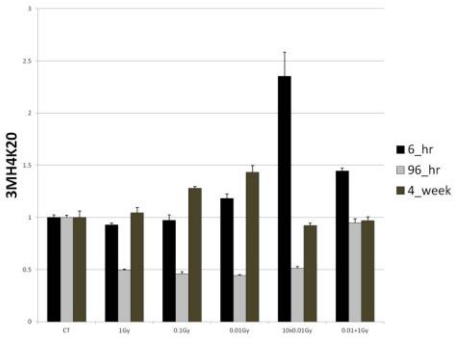


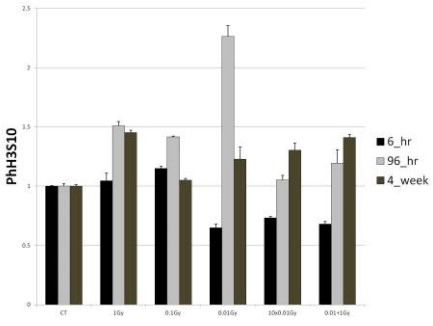










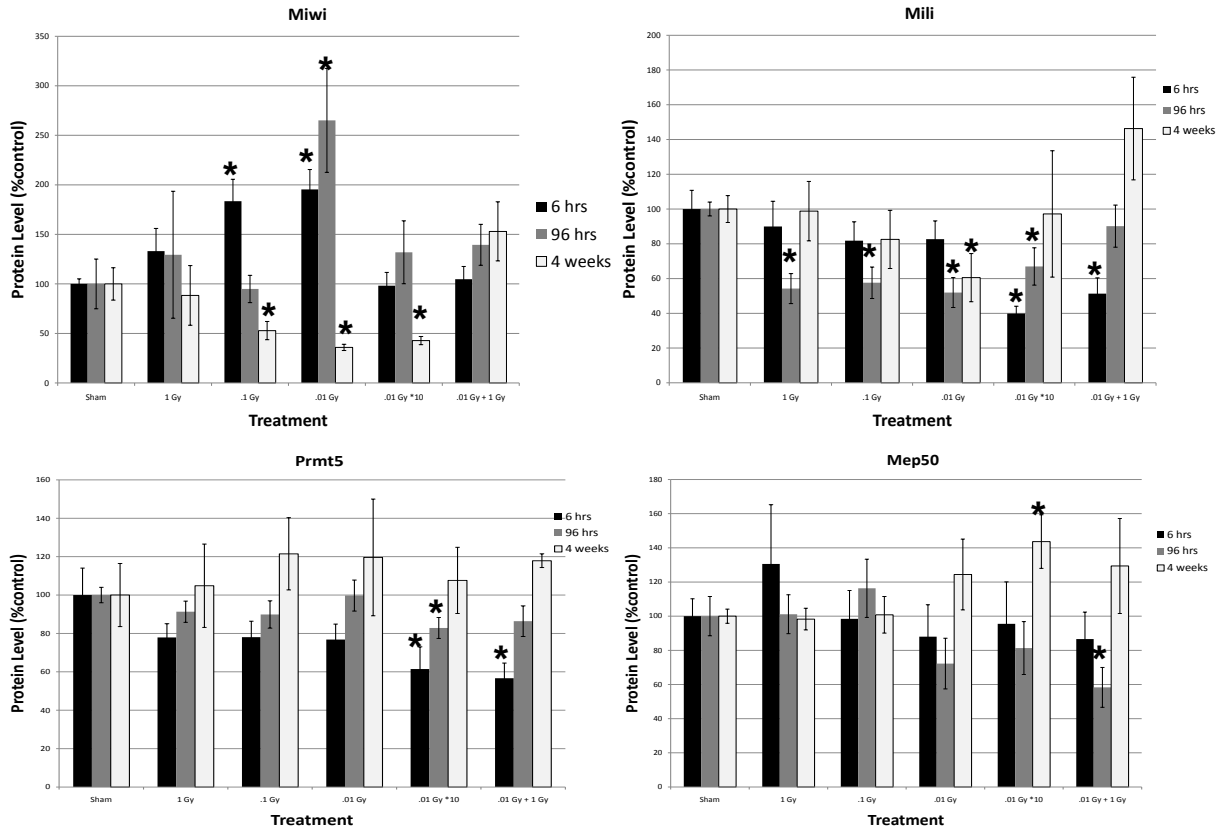


Changes observed in spleen were more profound than those observed in thymus.

GERMLINE

Having seen numerous epigenetic alterations in somatic tissues of mice, we analyzed changes in the germline.

We hypothesized that changes in epigenetic profiles (global and regional DNA methylation, chromatin status and regulatory small RNA pathways, e.g. piRNA) play pivotal roles in germline radiation responses. piRNAs and Piwi proteins facilitate a germline specific epigenetic regulatory mechanism essential for spermatogenesis, silencing of transposable elements, and maintaining germline genome integrity, yet their role in the response of the male germline to genotoxic stress remained unknown. We tested the hypothesis by using an established *in vivo* mouse model to assay for alterations in DNA methylation, piRNA pathway protein levels, and gene expression in the male germline after various exposures to X-rays including low, mid, high, fractionated and adaptive doses. **We found that Acute/fractionated low dose radiation induces alterations to piRNA pathway protein levels.**



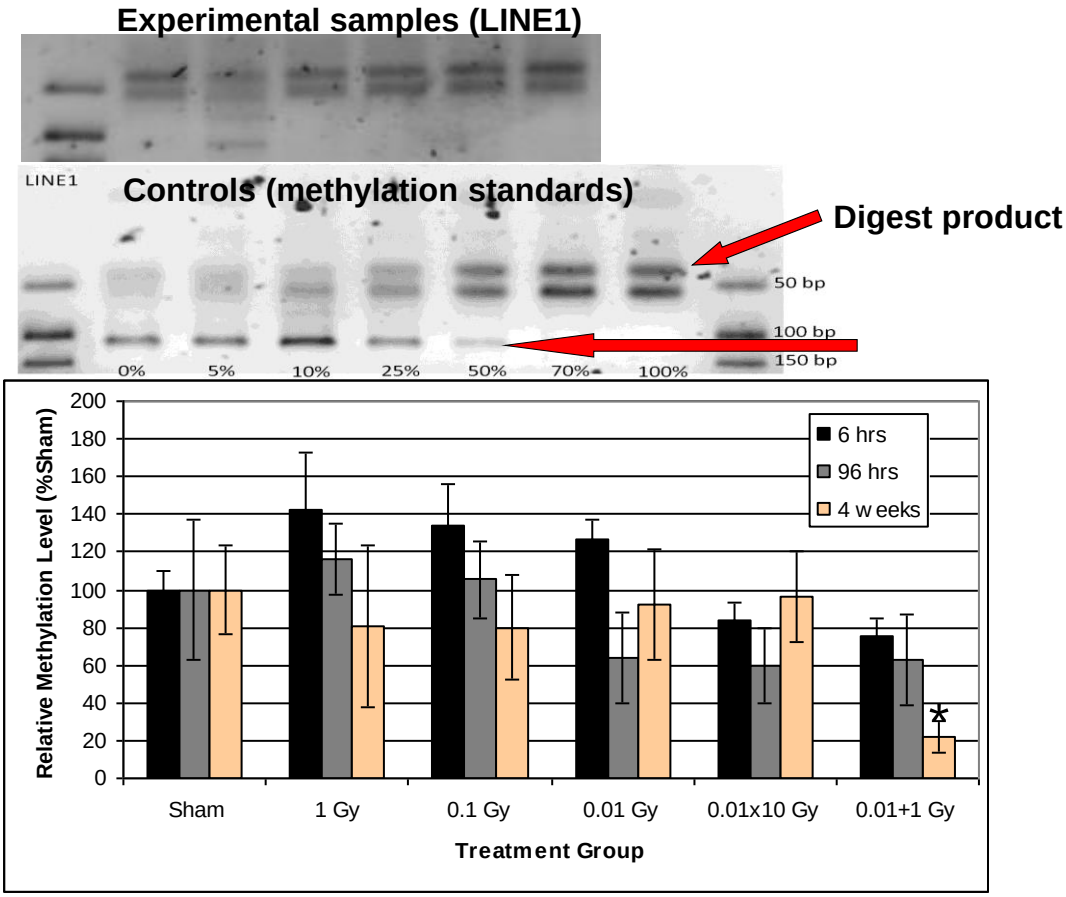
Miwi - Slicer protein (main protein component of piRNP complex)
Mili - Slicer protein (main protein component of piRNP complex)
Prmt5 - arginine methyltransferase (methylosome complex)
Mep50- Methyl binding protein? (methylosome complex)

Additionally, we noted significant gene expression changes following microarray analysis. Following pathway analysis, it was revealed that these genes most frequently belonged to oxidative phosphorylation processes (highlighted in green below).

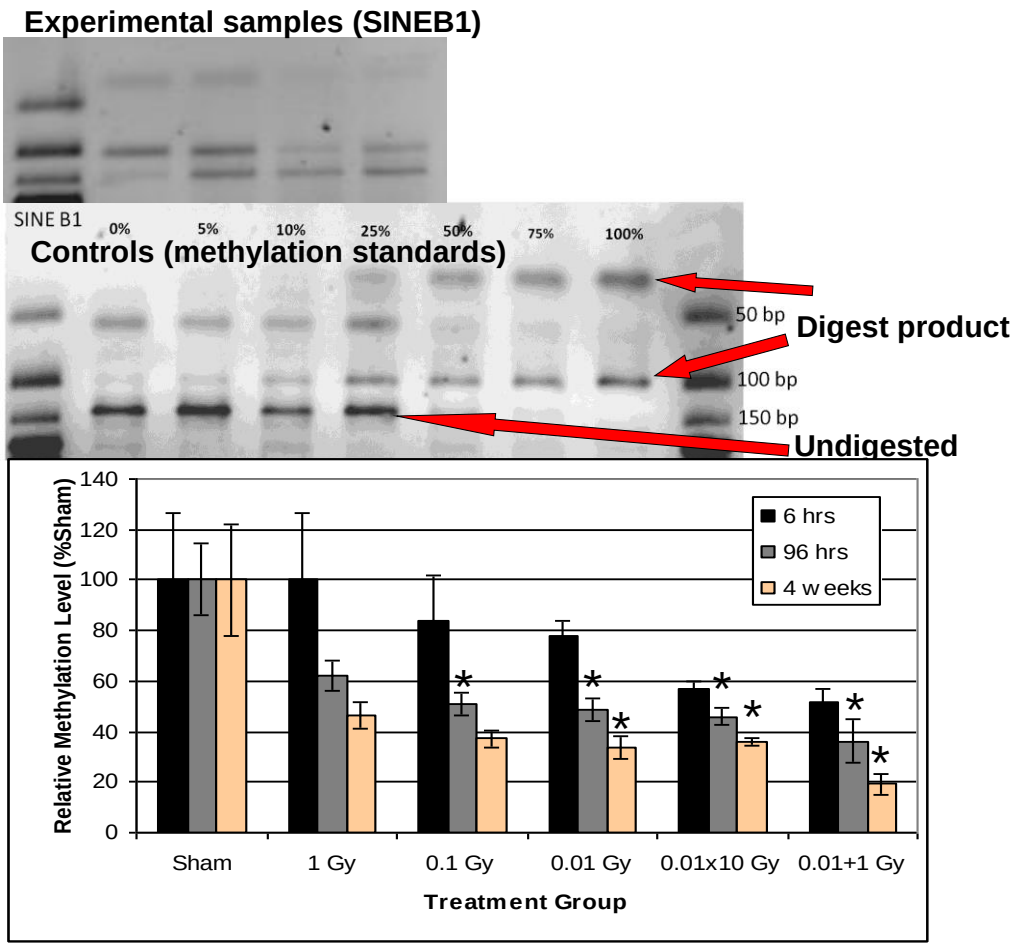
	1Gy	*0.1Gy*	*0.01Gy*	*0.01x10Gy*	*0.01+1Gy*
6hr	3 (all ↓)	1	40 (1 ↑: 39 ↓)	0	2 (all ↓)
96hr	0	11 (all ↓)	5 (4↑: 1↓)	278 (all ↓)	107 (all ↓)
4week	0	4 (all ↓)	1 (all ↑)	9 (1↑: 8↓)	15 (all ↓)

These changes were paralleled by alterations in the levels of DNA methylation of transposable elements LINE1 and SINEB2 as shown below.

COBRA Methylation Analysis of LINE1 Retrotransposon:



COBRA Methylation Analysis of SINEB2 Retrotransposon:



In sum, LDR exposure profoundly affected murine germline and caused genome instability and epigenetic changes.

PART II

DELAYED EFFECTS OF LDR EXPOSURE IN A MURINE MODEL

Animals received 0.1 Gy of X-rays and were sacrificed 55 weeks after exposure, whereby we analyzed delayed effects of LDR. We profiled changes in heart, spleen, liver and thymus of control and LDR-exposed animals.

LDR-induced changes in histone modification levels

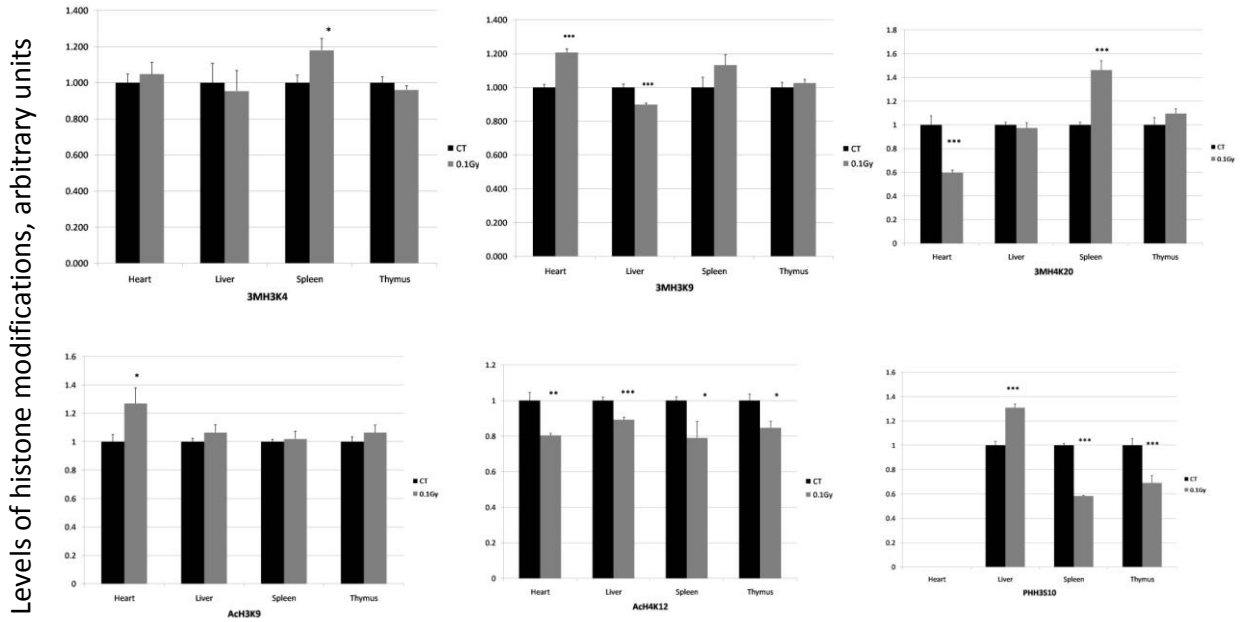
First we analyzed changes in several histone modifications using Western Blot analysis. Data summary is shown in a table below, whereby green denotes significant increases in histone levels, while red - significant decreases in histone modification levels.

3MH3K9	Heart			Liver			Spleen			Thymus		
	Fold	*St.Err*	*p-value*	*Fold*	*St.Err*	*p-value*	*Fold*	*St.Err*	*p-value*	*Fold*	*St.Err*	*p-value*
CT	1.000	0.018		1.000	0.020		1.000	0.059		1.000	0.029	
0.1Gy	1.207	0.022	0.000	0.898	0.009	0.004	1.133	0.061	0.171	1.026	0.021	0.539
3MH3K4												
CT	1.000	0.047		1.000	0.106		1.000	0.042		1.000	0.033	
0.1Gy	1.048	0.064	0.557	0.954	0.114	0.782	1.179	0.065	0.040	0.960	0.023	0.400
AcH3K9												
CT	1	0.05161		1	0.02493		1	0.01676		1	0.03318	
0.1Gy	1.26895	0.11029	0.03813	1.06571	0.05331	0.24536	1.02016	0.03571	0.582093149	1.06482	0.05967	0.33229
AcH4K12												
CT	1	0.04617		1	0.01838		1	0.02144		1	0.03734	
0.1Gy	0.80397	0.01072	0.00993	0.89207	0.01435	0.00295	0.79056	0.09136	0.026311631	0.84721	0.03698	0.02414
PHS10												
CT				1	0.0317		1	0.01687		1	0.05447	
0.1Gy				1.30903	0.03054	0.00016	0.58176	0.00569	5.17261E-08	0.6906	0.06022	0.00584
AcH4K8												
CT				1	0.04138		1	0.06219				
0.1Gy				1.2459	0.16714	0.08999	0.99003	0.06619	0.917881808			
3MH4K20												
CT	1	0.07472		1	0.02071		1	0.0203		1	0.05987	
0.1Gy	0.59497	0.025	0.00283	0.97311	0.04407	0.55278	1.4629	0.07863	0.000125215	1.09464	0.04119	0.27967
AcH4K5												
CT							1	0.11704				
0.1Gy							0.78193	0.08546	0.212967491			
PH3Ac												
CT							1	0.11176		1	0.0236	
0.1Gy							0.93779	0.07467	0.692439051	1.3429	0.06037	0.00029

1. 3MH3K9 - strong correlation with heterochromatin
    a. Heart = more silent, stable state of chromatin
    b. Liver = more open, expressive state
2. 3MH3K4 - Transcription activation
    a. Spleen = more transcription
3. AcH3K9 - Transcriptional activation and chromosome reassembly
    a. Heart = more transcription
4. AcH4K12 - present in activated genes but more in coding regions and not promoter regions; decreased acetylation at telomeres leads to more plastic, unstable telomeres
    a. Heart = less chromosome stability
    b. Liver = less chromosome stability
    c. Spleen = less chromosome stability
    d. Thymus = less chromosome stability

5. AcH4K8 - associated with overexpresed genes; decreased acetylation is linked to cell survival following damage
6. 3MH4K20 - highly enriched at peri-centromere, IAP retrosposons; increases with age; decreased acetylation associated with cancer progression
    a. Spleen - should have more stable genome
7. AcH4K5 - associated with active gene expression
8. PhH3S10 - counteracts heterochromatin spreading by blocking access of H3K9 modifying proteins
    a. Liver - less heterochromatin; more open state
    b. Spleen - more heterochromatin
    c. Thymus - more heterochromatin

Figure - Delayed effects of LDR on global histone modifications in different tissues of mice



LDR-induced gene expression changes

Despite the abundance of histone changes, there did not seem to be an equivalent manifestation of gene expression changes. No significant changes were noted in heart, spleen or thymus of LDR-exposed mice 55 weeks after exposure.

Liver had a few significant gene expression changes, however, most were not above the threshold limits of the detection platform (1.3 fold; log2 of +/- 0.38). Interestingly, one gene in liver, PIM3, was significant and outside the threshold limits. PIM3 is an oncogene.

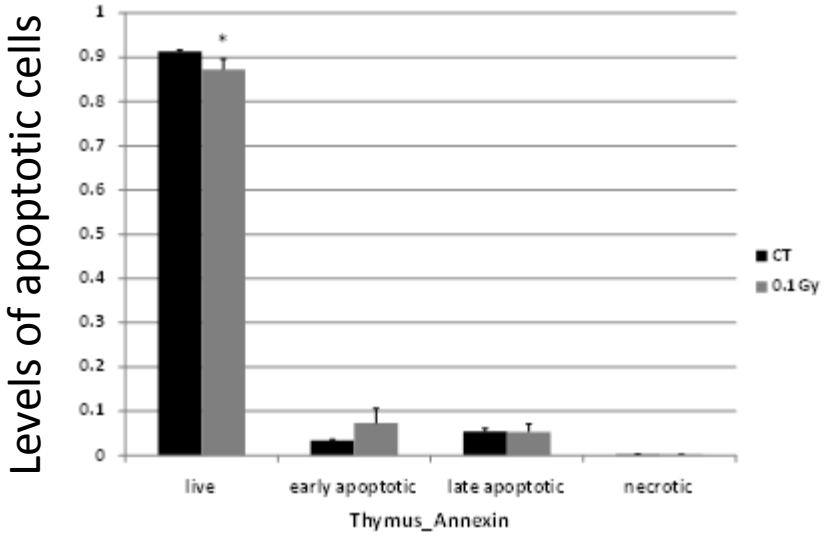
Gene	***fold***	***description***	***functions***
Apol9b	-0.33771	apolipoprotein L 9B	possible role in cholesterol transport; human homologues from the same L family have role in schizophrenia and exist on region of chromo 22 that is prone to deletions
Arpc3	-0.37486	actin related 2/3 complex	role in actin polymerization; highly conserved gene
Atp5g2	0.31798	and ATP synthase	
H2afz	0.315576	H2A histone variant	Seems to bee correlated w/ TSS displaying a high gene expression level, may also be involved

			in the spreading of heterochromatin (yeast)
Lman2	-0.3215	lectin mannose binding	cargo shuttling between the ER and Golgi
Pim3	0.541547	oncogene	expressed in all hepatomas and at regenerative nodes; not expressed in normal liver tissue, siRNA knock-downs severely retarded proliferation of hepatoma cells; knockdowns also increased apoptosis in pancreatic cancerous cell
Rpl12	0.324878	ribosomal 60S	associates with a protein that has role in G2/M transition and non sno-RNA splicing;

LDR-induced apoptosis changes

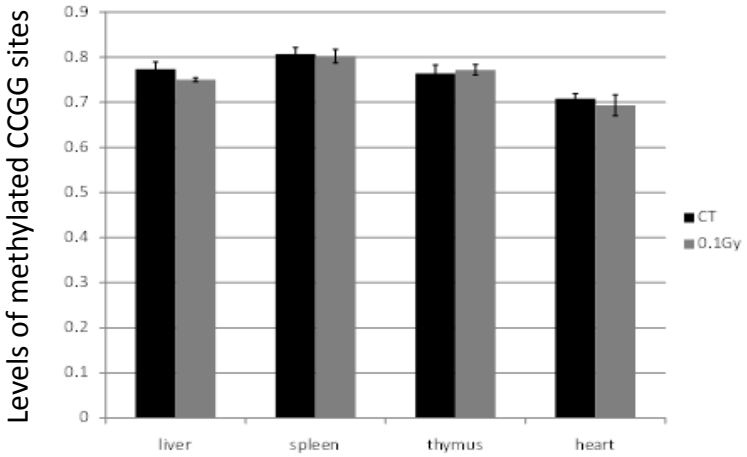
Apoptosis analysis did not reveal any major significant changes, except for a small decrease in the number of living cells in thymus of LDR-exposed mice.

Figure - Levels of apoptotic cells in thymus of LDR-exposed mice



LDR-induced global DNA methylation changes

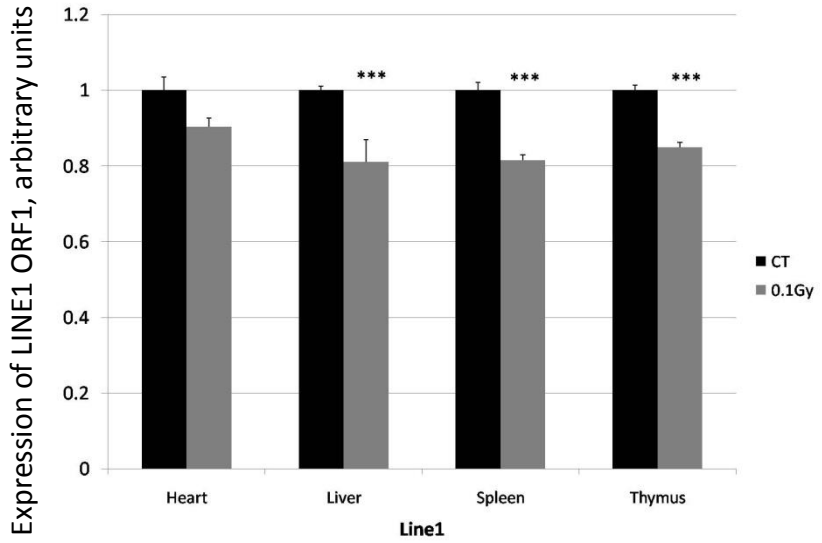
Global DNA methylation analysis was performed using a cytosine extension assay, and no significant differences between CT and exposed group were noted.



LDR-induced changes in the LINE1 expression levels

Even though global DNA methylation profiles seemed unchanged, all tissues actually demonstrated a decrease in LINE1 expression following the radiation exposure. These were highly significant in liver, spleen and thymus, however, barely significant (P=0.06) in heart. Increased levels of LINE1 expression are accepted markers of genome instability.

Figure – Levels of LINE1 ORF1 expression in tissues of LDR-exposed mice



<u>Very important conclusion:</u>

The decreased levels of LINE1 seen here may suggest an actual increase in the stability of the genomes over the long term after LDR exposure.

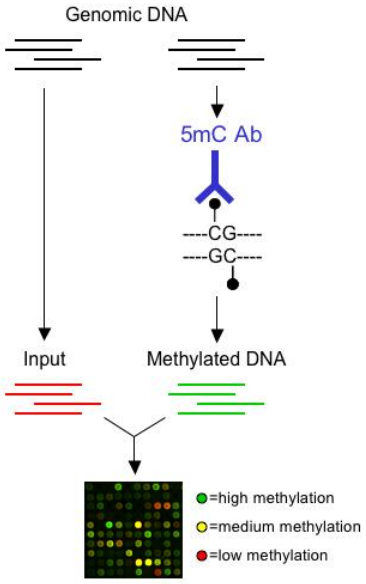
PART III
LDR AFFECTS GLOBAL DNA METHYLATION AND CAUSES MICRORNAOME CHANGES (0.05/0.5/5 GY)

We analyzed epigenetic effects in animals that received 0.05, 0.5 and 5 Gy of X-rays. In brief, fifty-day-old mice (15 males) were randomly assigned to different treatment groups. Five males received 0.05 Gy of X-ray exposure to the entire body (90 kV, 5 mA). Five animals received 5 Gy of X-ray exposure to the entire body (90 kV, 5 mA). Five were sham treated and served as control. Animals were sacrificed 24 hours after exposure. Spleens were harvested immediately following sacrifice, frozen in liquid nitrogen, and stored at -80 °C for further analysis.

The method for global DNA methylation as profiled using Methylated-DNA-Immunoprecipitation method (MeDIP) is presented on Figure 1.

Figure 1 – MeDIP method summary.

Me-DIP (methylated-DNA-immunoprecipitation) method



Principle of MeDIP (Methylated DNA Immunoprecipitation).
Total genomic DNA is sonicated and methylated DNA in immunoprecipitated with an antibody directed against 5-methylcytidine (5mC). Input DNA (IN) and methylated DNA (M) can be differentially labeled with Cy5 (red) and Cy3 (green) and co-hybridized as a two-color experiment on microarrays, or used for single-gene analysis by PCR.

http://www.epigenome-noe.net/researchtools/protocol.php?protid=33#fig

Analysis revealed a massive alteration in global DNA methylation profile upon LDR exposure. Loci that were differentially methylated in spleen tissue of animals exposed to low and high doses of radiation are shown on Figure 2 and Table 1.

Figure 2 - Loci that were differentially methylated in spleen tissue of animals exposed to low and high doses of radiation

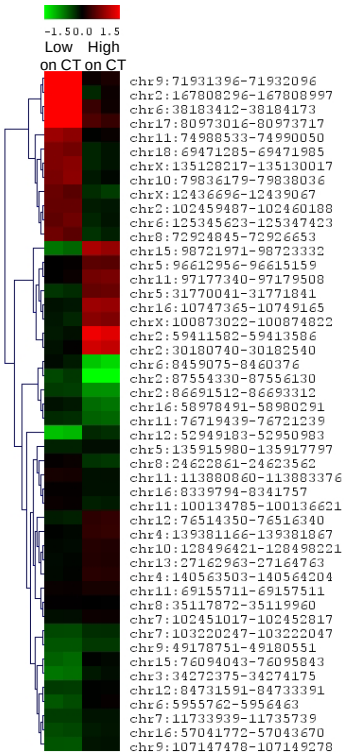


Table 1 – Loci that were differentially methylated in exposed animals.

SEQ_ID	CpG island	miR loci	Transcription start site	Primary Transcript	Biological Process of PrimaryTranscript	Related genes/pathway/network (literature based association)				
chr10:128496421-128498221	No	No	Olfr768	Olfr768	None	None				
chr10:79836179-79838036	Yes	No	TcfE2a	TcfE2a	Cell fate/differentiation	Tcf12	Tcf3	Atf3	Pax5	D3Ertd300e
chr11:100134785-100136621	Yes	No	Eif1	Eif1	Transcription factor	Akt1	Pim1	Pten	Cnot3	Jun
chr11:113880860-113883376	Yes	No	Sdk2	Sdk2	None	C1qtnf1	Hsp90b1	Sdk1	Hsp90ab1	
chr11:69155711-69157511	Yes	No	Pred.cDNA	Pred.cDNA	N/A					
chr11:74988533-74990050	Yes	miR-212, miR-132	None	None	N/A					
chr11:76719439-76721239	No	No	Tmigd	Tmigd	Trans-membrane protein	None				
chr11:97177340-97179508	Yes	No	Socs7	Socs7	Neg. regulator T-cell signalling	Socs4	Socs1	Socs3	Cish	Sg2
chr12:52949183-52950983	No	No	Gpr33	Gpr33	Neg.regulator Interferons	Mcr4	Gpr172b	Gpr151	Gpbar1	Abl2
chr12:76514350-76516340	Yes	No	Ppp2r5e	Ppp2r5e	Phosphatase	Ppp2r2b	Prnp	Cdr1	Cdr	
chr12:84731591-84733391	No	No	Numb	Numb	None	Notch1	Ubb	Dyrk1a	Msi1	Gpx6
chr13:27162963-27164763	No	No	Plig	Plig	Prolactin family	Selp	Ifng	Cd69	Cd44	Il2ra
chr15:76094043-76095843	No	No	Spatc1	Spatc1	Spindle checkpoint	Cdc20	Pred.cDNA			
chr15:98721971-98723332	Yes	No	None	Dhh	Spermidine metabolism	Sry	Sox9	Shh	Ptch1	Ihh
chr16:10747365-10749165	Yes	No	Pred.cDNA	Pred.cDNA	N/A	N/A				
chr16:57041772-57043670	Yes	No	Tomm709	Tomm709	Gene silencing	Akt1	Tomm20	Tomm22	Bcs1l	Gfm1
chr16:58978491-58980291	No	No	Olfr187	Olfr187	None	None				
chr16:8339794-8341757	Yes	No	Pred.cDNA	Pred.cDNA	N/A	N/A				
chr17:80973016-80973717	Yes	No	None	None	N/A	N/A				
chr18:69471285-69471985	Yes	No	None	Tcf4	Transcription factor	Tcf7l2	Hnf4a	Ctnnb1	D3Ertd300e	Atf4
chr2:102459487-102460188	Yes	No	None	None	N/A	N/A				
chr2:167808296-167808997	Yes	No	None	None	N/A	N/A				
chr2:30180740-30182540	Yes	No	Sh3gbl2	Sh3gbl2	None	None				
chr2:59411582-59413586	Yes	No	Tanc1	Tanc1	None	None				
chr2:86691512-86693312	No	No	Olfr1097	Olfr1097	None	None				
chr2:87554330-87556130	No	No	Olfr1191	Olfr1191	None	None				
chr3:34272375-34274175	Yes	No	Dnajc19	Dnajc19 (X2)	Heat Shock protein	None				
chr4:139381166-139381867	Yes	No	None	Igsf21	Immunoglobulin super family	None				
chr4:140563503-140564204	Yes	No	None	None	N/A	N/A				
chr5:135915980-135917797	Yes	No	Rhbdd2	Rhbdd2	None	None				
chr5:31770041-31771841	Yes	No	Xab1	Xab1	XPA binding, DNA methylation	Mbd2				
chr5:96612956-96615159	Yes	No	Fras1	Fras1	Development	Lgals12				
chr6:125345623-125347423	Yes	No	Plkhg6	Plkhg6	None	None				
chr6:38183412-38184173	Yes	No	None	None	N/A	N/A				
chr6:5955762-5956463	Yes	No	None	Dync1i1	Post-translat. protein mods.	Myo1e	Myo1b	Mapre3	Chia	Myo7a
chr6:8459075-8460376	Yes	No	None	Glcci1	Differentiation	Sp3	Sp1	Tssn1	Cd4	
chr7:102451017-102452817	No	No	Olfr550	Olfr550	None	None				
chr7:103220247-103222047	No	No	Olfr600	Olfr600	None	None				
chr7:11733939-11735739	Yes	No	Zfp110	Zfp110	Neural differentiation	Ngfr	Tnfrsf	Zfp369	Prdm4	
chr8:24622861-24623562	Yes	No	None	None	N/A	N/A				
chr8:35117872-35119960	Yes	No	Gsr	Gsr	Blood differentiation	Ly6g	Itgam	Emr1	Cd4	Ifng
chr8:72924845-72926653	Yes	No	Gatad2a	Gatad2a	Development	Gatad2b (HD)	Shc1	Mbd2		
chr9:107147478-107149278	No	No	Mapkapk3	Mapkapk3	Signaling	Kcna3	Kcna2	Map2k2	Mapkapk2	Crk (v-oncogene)
chr9:49178751-49180551	No	No	Ankk1	Ankk1	None	None				
chr9:71931396-71932096	Yes	No	None	None	N/A	N/A				
chrX:100873022-100874822	Yes	No	Zdhhc15	Zdhhc15	None	None				
chrX:12436696-12439067	Yes	No	Ddx3x	Ddx3x	Nervous development	Dhx9	D1Pas1	Dhx8	Ddx56	Ddx3y
chrX:135128217-135130017	No	No	Ripply1	Ripply1	Development	None				

Next we analyzed the effects of LDR on cellular microRNAome. We found that radiation exposure caused alterations in levels of various microRNAs and affected the levels of microRNA targets, such as a key regulator E2F3 (Figure 3).

Moreover, radiation exposure affected microRNA processing machinery in murine spleen and altered expression of Dicer, Ago, PACT, TudorSN and FxR proteins (Figure 4). Dicer is a ribonuclease that cleaves the pre-miRNA, leading to the formation of a mature miRNA. To control translation of target mRNAs, mature miRNAs must associate with RNA-induced silencing complex (RISC) proteins such as Argonaute (Ago), PACT (a protein activator of the interferon-induced protein kinase), fragile X mental retardation protein (FxR), TudorSN or other proteins. MicroRNAs, associated with RISCs, bind to a specific section of mRNAs, thus serving as translational suppressors and regulating the production of proteins.

Figure 3 – Radiation-induced changes in microRNAome of the spleen tissues of radiation-exposed mice

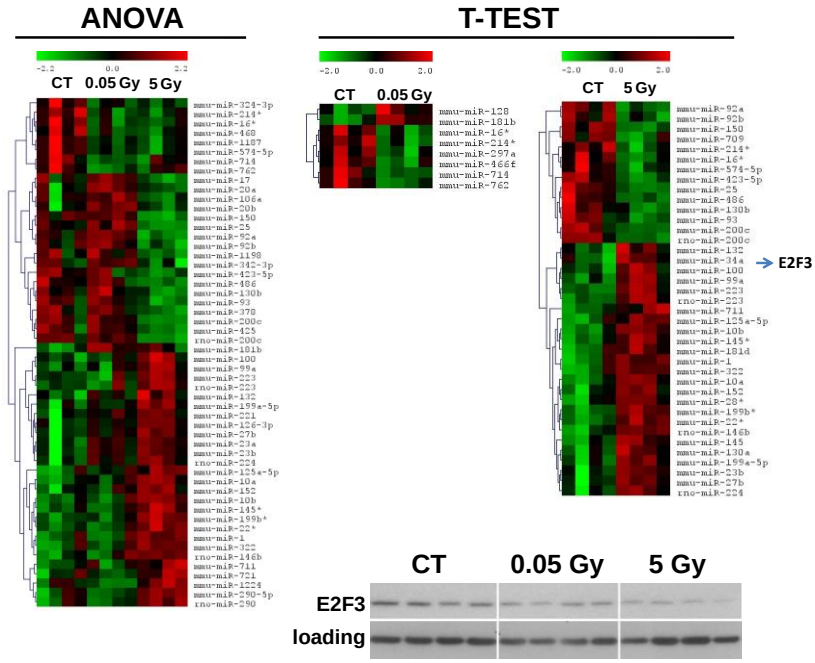
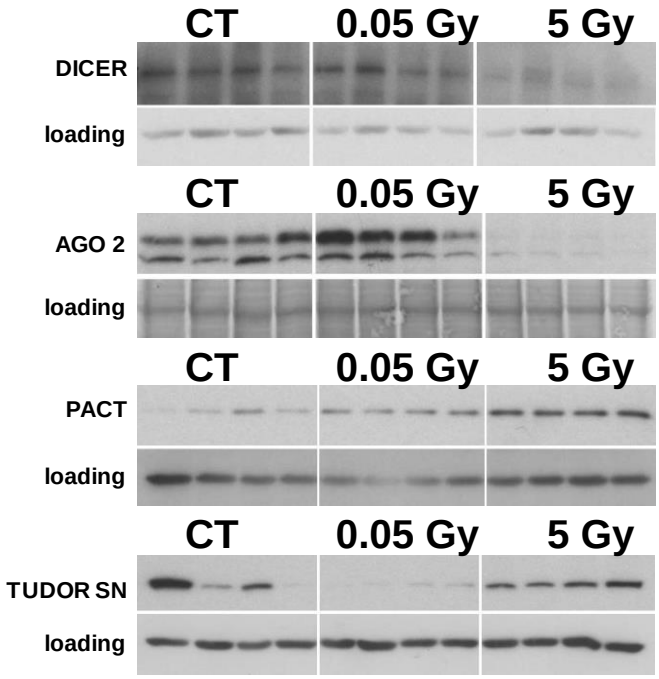


Figure 4 - Radiation exposure affects microRNA processing machinery



PART IV

EFFECTS OF LDR ON MAMMARY GLAND TISSUES – ABSTRACTS OF 3 PAPERS THAT WILL BE SUBMITTED FOR PUBLICATION IN FALL 2014.

Low dose irradiation profoundly affect transcriptome and microRNAme in rat mammary gland tissues: implications for carcinogenesis

Ionizing radiation has been successfully used in medical tests and treatment therapies for a variety of medical conditions. However, patients and individuals providing patient care are greatly concerned about overexposure to medical ionizing radiation and possible cancer induction due to frequent mammographies and/or CT scans. Diagnostic imaging involves the use of low doses of ionizing radiation, and its potential carcinogenic role creates a cancer risk concern for exposed individuals. In this study, the effects of X-ray exposure of different doses on the gene expression patterns and the micro-RNA expression patterns in normal breast tissue were investigated in rats. Our results revealed the activation of immune response pathways upon low doses of radiation exposure. These included natural killer cell-mediated cytotoxicity pathways, antigen processing and presentation pathways, chemokine signaling pathways, and T- and B-cell receptor signaling pathways. Both high and low doses of radiation led to miRNA expression alterations. Increased expression of miR-34a may be linked to cell cycle arrest and apoptosis. Up-regulation of miR-34a was correlated with down-regulation of its target E2F3 and up-regulation of p53. This data suggests that ionizing radiation at specific high and low doses leads to cell cycle arrest and a possible initiation of apoptosis.

Mobilization of LINE-1 in irradiated mammary gland tissue may potentially contribute to low dose radiation-induced genomic instability

It is known that cellular stresses such as ionizing radiation activate LINE-1 (long interspersed nuclear element type 1, L1), but the molecular mechanisms of LINE-1 activation have not been fully elucidated. There is a possibility that DNA methylation changes induced by genotoxic stresses might contribute to LINE-1 activation in mammalian cells. L1 insertions usually cause

major genomic rearrangements, such as deletions, transductions, the intrachromosomal homologous recombination between L1s, and the generation of pseudogenes, which could lead to genomic instability. The purpose of this study was to evaluate the effects of low and high doses of ionizing radiation on the DNA methylation status of LINE-1 transposable elements in rat mammary glands. Here we describe radiation-induced hypomethylation and activation of LINE-1 ORF1 in rat mammary glands. Radiation exposure has also led to the translation of the LINE-1 element. A 148 kDa LINE-1 protein level was increased 96 hours after treatment with a low energy level and a low dose and remained high for 24 weeks after treatment. The mobilization of LINE-1 in irradiated tissue potentially contributes to genomic instability and cancer initiation. The activation of mobile elements in response to radiation exposure is consistently discussed as a plausible mechanism of cancer etiology and development.

High and low dose radiation effects on mammary adenocarcinoma cells – and epigenetic connection

The successful treatment of cancer, including breast cancer, depends largely on radiation therapy and proper diagnostics. The effect of ionizing radiation on cells and tissues depends on the radiation dose and energy, but there is insufficient evidence concerning how tumor cells respond to the low and high doses of radiation that are often used in medical diagnostic and treatment modalities. The purpose of this study was to investigate radiation-induced gene expression changes in the MCF-7 breast adenocarcinoma cell line. Using microarray technology tools, we were able to screen the differential gene expressions between various radiation doses applied to MCF-7 cells. Here, we report the substantial alteration in the expression level of genes after high dose treatments. In contrast, no dramatic gene expression alterations were noticed after the application of low and medium doses of radiation. In response to a high radiation dose, MCF-7 cells exhibited down-regulation of biological pathways such as cell cycle, DNA replication, and DNA repair and activation of the p53 pathway. Similar dose-dependent responses were seen on the epigenetic level, tested by a microRNA expression analysis. MicroRNA analysis showed dose-dependent radiation-induced microRNA expression alterations that were associated with cell cycle arrest and cell death. An increased rate of apoptosis was determined by an AnnexinV assay. The results of this study showed that high doses of radiation affect gene expression genetically and epigenetically, leading to alterations in cell cycle, DNA replication, and apoptosis.

EFFECTS OF LDR ON GLIOBLASTOMA AND NEUROBLASTOMA CELLS

Cells were exposed to 0.1 Gy of X-rays and gene expression and DNA methylation profiles were analyzed 24 and 72 hours after exposure.

Gene-specific DNA methylation and gene expression changes induced by low dose radiation in human neuroblastoma (A-172 and IMR-32) and glioma cells (SK-N-BE)

DNA methylation			
	A-172	**IMR-32**	**SK-B-NE**
24 hours	19	3	17
72 hours	90	1358	9
Gene expression			
	A-172	**IMR-32**	**SK-B-NE**
24 hours	113	225	2
72 hours	3	4	0

Correlation between the levels of gene expression, methylation and apoptosis in the studied neuroblastoma and glioma cells

DNA methylation			
	A-172	**IMR-32**	**SK-B-NE**
24 hours	+	+	++
72 hours	+++++++	++++++++++++++++++++++	+
Gene expression			
	A-172	**IMR-32**	**SK-B-NE**
24 hours	++++	+++++++++	+
72 hours	+	++	-
Apoptosis			
	A-172	**IMR-32**	**SK-B-NE**
24 hours	+	++	-
72 hours	++	+++++	- -

SAMPLE OF A SPIN-OFFPROJECT BASED ON PROFILING RESULST

A SUPPRESSIVE ROLE OF IONIZING RADIATION-RESPONSIVE MIR-29C IN LIVER CARCINOGENESIS VIA TARGETING WIP1 (SUBMITTED TO ONCOTARGET)

Being the third most common cause of cancer-related death worldwide, hepatocellular carcinoma (HCC) has been linked with radiation exposure. As a well-defined oncogene, wild-type p53-induced phosphotase 1 (WIP1) plays an inhibitory role in several tumor suppressor pathways, including p53. WIP1 has been demonstrated to be amplified and overexpressed in many malignancies including HCC. The underlying mechanisms, however, remain largely unknown. Our study shows that low-dose ionizing radiation (IR) remarkably induces miR-29c expression in female mouse liver, while inhibits its expression in Hep G2, a human hepatocellular carcinoma cell line, used as a model system here. miR-29c expression is downregulated in human hepatocellular carcinoma cells, that is inversely correlated with WIP1 expression. miR-29c attenuates luciferase activity of a reporter harboring the 3'UTR binding motif of WIP1 mRNA. Ectopic expression of miR-29c significantly represses cell proliferation, induces apoptosis and G1 arrest in Hep G2. In contrast, the knockdown of miR-29c remarkably enhances Hep G2 cell proliferation and suppresses apoptosis. The biological effects of miR-29c mimic and inhibitor may be mediated by its target WIP1 that regulates p53 activity via dephosphorylation at Ser15. Finally, FISH and immunohistochemical analyses indicate that miR-29c is downregulated in 50.6% of liver carcinoma tissues examined, whereas WIP1 is upregulated in 45.4% of these tissues. The expression of miR-29c correlates well with that of WIP1 in HCC. Our results suggest that the IR-responsive miR-29c may function as a tumor suppressor that plays a crucial role in the development of liver carcinoma via targeting WIP1 and may therefore represent a target molecule for therapeutic intervention for this disease.

DISSEMINATION OF RESULTS

Five articles are now being written based on the results presented in the report.

SUBMITTED

1. B. Wang, D. Li, C. Sidler, R. Rodriguez-Juarez, N. Singh, M. Heyns, R. T. Bronson, O. Kovalchuk. A suppressive role of ionizing radiation-responsive miR-29c in liver carcinogenesis via targeting WIP1. ***Oncotarget*** (submitted)

ARTICLES IN REFEREED JOURNALS PUBLISHED OR IN PRESS:

2. B. Wang, D. Li, A. Kovalchuk, D. Litvinov, **O. Kovalchuk**. 2014. Ionizing radiation-inducible miR-27b suppresses leukemia proliferation via targeting cyclin A2. ***International Journal of Radiation Oncology*Biology*Physics*** 90:53-62.

3. M. Merrifield, **O. Kovalchuk**. 2014. Epigenetics in radiation biology: a new research frontier. ***Front Genet***. 4:40. doi: 10.3389/fgene.2013.00040

4. Y. Ilnytskyy, **O. Kovalchuk**. 2011. Non-targeted radiation effects-An epigenetic connection. ***Mutat Res***. 714:113-25

EDITED BOOKS:

"Epigenetics of Health and Disease" by Pearson Inc, I. Kovalchuk and **O. Kovalchuk**. Pub. Date: 2012.

"O. Kovalchuk (2012) Epigenetic Effects of Ionizing Radiation. In "Environmental Epigenomics in Health and Disease*" by Springer Inc.*

PRESENTATIONS AS AN INVITED/GUEST SPEAKER:

RESEARCH PRESENTATIONS:

1. Epigenetic regulation of the cancer treatment responses. 13th International Workshop on Radiation Damage to DNA Cambridge, Massachusetts, June 14-18, 2014. *Invited workshop speaker.*

2. Ying and Yang of radiation epigenetics. Children's Dmitry Rogachev Oncology Center, Moscow, Russia, May 2014. *Invited speaker.* Host – Dr. Sergey Roumiantsev.

3. Ying and Yang of radiation epigenetics. Summer School of Radiation Biology, Obninsk, Russia, May 2014. *Invited speaker.*

4. Ying and Yang of radiation biology. Children's Dmitry Rogachev Oncology Center, Moscow, Russia, June 2013. *Invited speaker.* Host – Dr. Sergey Roumiantsev.

5. Radiation and brain: knowns and unknowns. CCBN summer Speaker Series, May 2013, Lethbridge, AB.

6. Radiation epigenetics. 2012 Alberta Cancer Foundation Cancer Research Conference, November 2012, Banff, Alberta. *Invited speaker.*

7. Role of Epigenetic Deregulation in Radiation-Induced Genome Instability and Carcinogenesis. The 58th Annual Meeting of the Radiation Research Society. September 30th-October 3rd, 2012, San Juan, PR. *Invited speaker.*

8. Radiation-induced (epi)genome instability. MELODI Low Dose Research Platform Workshop. September 12-14th, 2012, Helsinki, Finland. *Invited speaker*.

9. Epigenetic Changes in Radiation-Induced Genome Instability and Carcinogenesis. April, Kiev Institute of Experimental Oncology, Kiev, Ukraine, April, 2012. *Invited speaker.* Host - Dr. Vasyl Chekhun.

10. Epigenetic Changes in Radiation-Induced Genome Instability and Carcinogenesis. The 55th Annual Meeting and Conference on Epigenetics and Genomic Stability, Whistler, BC, March 14th-18th, 2012. *Invited symposium speaker.*

11. Small RNAs in radiation responses. University of Hawaii at Manoa, February 23, 2012. *Guest speaker*. Host – Dr. Stefan Moysiadi.

12. Radiation Epigenetics – Power and Promise. Cross Cancer Institute, Oncology Rounds, Edmonton, February 2nd, 2012. *Invited speaker*.

13. Radiation epigenetics – power and promise. STUK – Finnish Radiation and Nuclear Safety Authority, Helsinki, October 10th, 2011. *Invited speaker*. Host – Dr. Sisko Salomaa.

14. Epigenetic changes in radiation-induced genome instability and carcinogenesis. 8th Valamo Conference on Environment and Health, Valamo monastery, Heinävesi, Finland, October 10-12, 2011. *Keynote speaker.*

15. Radiation induced epigenetic changes. 14th International Congress of Radiation Research, Warsaw, Poland, August 28 - September 1, 2011. *Invited speaker.*

16. Radiation epigenetics – a story of two generations. Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia, August 25th, 2011. *Invited speaker.* Host – Dr. Anton Buzdin.

17. Epigenetic Changes as Biomarkers of Low Dose Radiation Exposure: Power and Promise. Low Dose Radiation Research Program Investigators' Workshop, Bethesda, MD, May 9-11, 2011. *Invited speaker.*

18. Epigenetic changes in radiation-induced genome instability and carcinogenesis. Epigenetics, Eh! Canadian Conference on Epigenetics, London, On, May 2-4, 2011. *Invited speaker.*

19. Role of small RNAs in radiation responses and radiation-induced genome instability. Society of Toxicology of Canada, Montreal, Quebec, December 6-7, 2010. *Invited speaker.*

20. MicroRNAome Changes in Radiation-Induced Genome Instability. EMS 41st Annual Meeting 2010: Omni Fort Worth Hotel, Fort Worth, Texas, October 23–27, 2010. *Invited speaker.*

21. Epigenetics of radiation-induced genome instability. NATO ARW, Alushta, Ukraine, October 9-14, 2010. *Invited speaker.*

22. Role of Epigenetic Deregulation in Radiation-Induced Genome Instability and Carcinogenesis. The 2009 Annual Meeting of the Environmental Mutagen Society, St. Louis, USA, October 24-28, 2009. *Invited speaker - symposium presentation.*

23. Epigenetic mechanisms of abscopal/bystander effects in an animal model. Presidential symposium, Annual Radiation Research Society Meeting, Savannah, GA, USA, October 5-9, 2009. *Invited speaker – Presidential symposium presentation.*

24. Role of epigenetic deregulation in radiation-induced genome instability and carcinogenesis. Symposium "Epigenome and the environment: from understanding the mechanisms to risk assessment', the 10th ICEM Meeting, Florence, Italy, 2009. *Invited speaker - symposium presentation.*

25. Epigenetic deregulation in radiation-induced genome instability. Department of Genetics, University of Leicester, UK, 2009.

26. Role of epigenetic dysregulation in radiation-induced genome instability. Symposium "Radiation molecular genetics" at ARR Annual Meeting, Glasgow, UK, 2009.

27. Role of epigenetic deregulation in radiation-induced genome instability. National Cancer Institute, NIH, 2008. Host – Dr. William Bonner.

28. Role of Epigenetic Deregulation in Targeted and Non-Targeted Radiation Responses. The 2007 Annual Meeting of the Environmental Mutagen Society, San Juan, Puerto Rico, October 18-22, 2008. *Invited speaker - symposium presentation.*

29. In vivo radiation exposure effects: epigenetic mechanisms of radiation-induced transgenerational genome instability. Symposium "Genetic Effects and Carcinogenesis at Low Dose Radiation Exposures", October 6-8, 2008, Rokkasho, Japan. *Invited speaker - symposium oral presentation* (gratefully declined).

30. Role of epigenetic deregulation in the non-targeted radiation effects. National Institute of Radiological Sciences (NIRS), Chiba, Japan, June 6, 2008. Host - Dr. Kazuo Sakai.

31. Epigenetics of the non-targeted radiation effects. Institute of Biotechnology, Tsukuba, Japan, June 5, 2008. Host – Dr. Seiichi Toki.

32. Epigenetic deregulation in radiation carcinogenesis. Alberta Genomic Instability and Aging Conference, April 30-May 1, 2008, Calgary, AB. Invited speaker.

33. Epigenetic dysregulation in radiation carcinogenesis, University of Georgia, USA, October 25, 2007. Host – Professor William Dynan.

34. Epigenetic dysregulation in estrogen- and radiation-induced carcinogenesis. Institute of Experimental Oncology, Kiev, Ukraine, September 13, 2007. Host- Professor V. Chekhun.

ORAL PRESENTATIONS BY GROUP MEMBERS:

1. Igor Koturbash & **Olga Kovalchuk**. Epigenetics of Non-Targeted Radiation Effects. The 38th Annual EMS Meeting is Mutational and Epigenetic Mechanisms of Susceptibility and Risks for Genetic Diseases, Atlanta, GA, October 20-24, 2007. ***Symposium presentation***.

2. Jan Tamminga, **Olga Kovalchuk**. Epigenetic signature of radiation exposure in the male germline. The 38th Annual EMS Meeting is Mutational and Epigenetic Mechanisms of Susceptibility and Risks for Genetic Diseases, Atlanta, GA, October 20-24, 2007. ***Platform presentation***.

ABSTRACTS OF THE CONFERENCE PRESENTATIONS (NAMES OF PRESENTERS ARE UNDERLINED):

1. A.Kovalchuk, R.Mychasiuk, A. Muhammad, S. Hossain, S. Ilnytskyy, C. Kirkby, E.Ghasroddashti, **O. Kovalchuk**, B. Kolb. Novel insights into radiation-induced bystander effects in brain. The Alberta Cancer Foundation Cancer Research Conference 2013, Banff, Alberta, October 20-22, 2013.

2. A. Kovalchuk, R.Mychasiuk, A. Muhammad, S. .Hossain, S. Ilnytskyy, C. Kirkby, E.Ghasroddashti, **O.Kovalchuk**, B. Kolb. Novel insights into radiation-induced bystander effects in brain. 5th Alberta Genomic Instability & Aging Research Symposium, the Southern Alberta Cancer Research Institute University of Calgary, Calgary, Alberta, Canada September 5, 2013.

3. A. Kovalchuk, R. Rodriguez-Juarez, B. Kolb and **O. Kovalchuk**. ANALYSIS OF MICRORNAOMES IN RADIATION-SENSITIVE & RADIATION-RESISTANT GLIOBLASTOMA CELLS. Alberta Cancer Foundation Cancer Research Conference, Banff, Alberta, November 2012.

4. A. Kovalchuk, J. Novak, R.Rodriguez-Juarez, B. Kolb, **O. Kovalchuk**. Radiation effects on neuroblastoma and glioblastoma –an epigenetic connection. Alberta Cancer Foundation Cancer Research Conference, Banff, Alberta, November 2012.

5. **O. Kovalchuk**. Role of Epigenetic Deregulation in Radiation-Induced Genome Instability and Carcinogenesis. . The 58th Annual Meeting of the Radiation Research Society. San Juan, PR, USA, September 30th-October 3rd, 2012.

6. A. Kovalchuk, R. Mychasiuk, A. Muhammad, S. Ilnytskyy, B. Kolb, **O.Kovalchuk**. NOVEL INSIGHTS INTO RADIATION-INDUCED BYSTANDER EFFECTS IN BRAIN. . The 58th Annual Meeting of the Radiation Research Society. September 30th-October 3rd, 2012, San Juan, PR. The 58th Annual Meeting of the Radiation Research Society. San Juan, PR, USA, September 30th-October 3rd, 2012.

7. A. Kovalchuk, R. Rodriguez-Juarez, B. Kolb and **O. Kovalchuk**. ANALYSIS OF MICRORNAOMES IN RADIATION-SENSITIVE & RADIATION-RESISTANT GLIOBLASTOMA CELLS. The 58th Annual Meeting of the Radiation Research Society San Juan, PR, USA, September 30th-October 3rd, 2012.

8. **O. Kovalchuk**. Role of Epigenetic Deregulation in Radiation-Induced Genome and Epigenome Instability. MELODI Low Dose Research Platform Workshop. September 12-14th, 2012, Helsinki, Finland.

9. **O. Kovalchuk.** Epigenetic changes in radiation-induced genome instability and carcinogenesis. The 55th Annual Meeting and Conference on Epigenetics and Genomic Stability, Whistler, BC, March 14th-18th, 2012. Invited symposium speaker.

10. **O. Kovalchuk**. Epigenetic changes in radiation-induced genome instability and carcinogenesis. 8th Valamo Conference on Environment and Health, Valamo monastery, Heinävesi, Finland, October 10-12, 2011. Keynote speaker.

11. **O. Kovalchuk.** Radiation induced epigenetic changes. 14th International Congress of Radiation Research, Warsaw, Poland, August 28 - September 1, 2011. Invited plenary presentation.

12. A. Kovalchuk, J. Novak, R. Rodriguez-Juarez, B. Kolb and **O. Kovalchuk**. Brain tumors – an epigenetic connection: analysis of epigenetic changes in radiation-sensitive and radiation-resistant glioblastoma cells. 14th International Congress of Radiation Research, Warsaw, Poland, August 28 - September 1, 2011.

13. **O. Kovalchuk**. Epigenetic changes in radiation-induced genome instability and carcinogenesis. Epigenetics, Eh! Canadian Conference on Epigenetics, London, On, May 2-4, 2011. Invited symposium presentation.

14. J. Filkowski, K. Areshenko, S. Ilyntskyy and **O. Kovalchuk**. Analysis of low dose radiation induced epigenetic modifications in animal and human cell line models. Low Dose Radiation Research Program Investigators' Workshop, Bethesda, MD, May 9-11, 2011.

15. M. Merrifield, J. Filkowski, Ol. Kovalchuk. Epigenetic responses in murine germline after low dose radiation exposure. Low Dose Radiation Research Program Investigators' Workshop, Bethesda, MD, May 9-11, 2011.

16. **O. Kovalchuk**. Epigenetic changes in radiation-induced genome instability and carcinogenesis. Epigenetics, Eh! Canadian Conference on Epigenetics, London, On, May 2-4, 2011. Invited symposium presentation.

17. **O. Kovalchuk.** Role of small RNAs in radiation responses and radiation-induced genome instability. Society of Toxicology of Canada, Montreal, Quebec, December 6-7, 2010. Invited speaker.

18. J. Filkowski, N. Singh, D. Litvinov and **O. Kovalchuk**. Epigenetics of radiation-induced instability. Radiobiological Issues Pertaining to Environmental Security and Ecoterrorism. Advanced Research Workshop, Alushta, Ukraine, 9-14 October 2010, Invited Plenary Talk.

19. J. Filkowski and **O. Kovalchuk**. Radiation-induced epigenetic changes in an animal model. Cancer Genomics, Epigenomics, and the Development of Novel Therapeutics. February 5-9, 2010, Waikoloa, HI, USA.

20. J. Filkowski, I. Koturbash and **O. Kovalchuk**. Analysis of radiation-induced epigenetic changes in an animal model. The 3rd Annual Alberta Cancer Research Institute Research Meeting, November 2009, Banff, AB.

21. **O. Kovalchuk**. Role of epigenetic deregulation in radiation-induced genome instability and carcinogenesis. EMS 40th Annual Meeting is "Genomics in the Environmental Century", St. Louis, Missouri, USA, October 24-28, 2009.

22. **O. Kovalchuk**. Role of epigenetic deregulation in radiation-induced genome instability and carcinogenesis. The 10th ICEM "The Renaissance of Environmental Mutagenesis", Firenze (Florence, Italy), August 20-25, 2009.

23. J. Filkowski, Y. Ilnytsky, J. Tamminga, and **O. Kovalchuk**. DNA methylation and microRNAome deregulation underlie transgeneration radiation-induced genome instability. Keystone Symposium "The Biology of RNA Silencing", Victoria, BC, April 25 - 30, 2009.

24. J. Filkowski, I. Koturbash and **O. Kovalchuk**. Analysis of radiation-induced epigenetic changes in an animal model. Low Dose Radiation Research Program Workshop, Bethesda, MD, April 6-8, 2009.

25. **O. Kovalchuk**. Role of epigenetic deregulation in targeted and non-targeted radiation responses. 39th Annual Meeting of the Environmental Mutagen Society "Genes and the Environment: From Molecular Mechanisms to Risk", Puerto Rico, October 18-22, 2008.

26. **O. Kovalchuk**. Role of epigenetic changes in radiation-induced bystander effects in vivo. 10th International Workshop on Radiation Damage to DNA, June 8-12, 2008, Fukushima, Japan.

27. **O. Kovalchuk**. Epigenetic deregulation in radiation carcinogenesis. Alberta Genomic Instability and Aging Conference, April 30-May 1, 2008, Calgary, AB.