Profiling ATM regulated genes in Drosophila at physiological condition and after ionizing radiation

Jun Liu¹, Tianyu Jin², Lanxi Ran², Ze Zhao², Rui Zhu², Gangcai Xie¹* and Xiaolin Bi¹,2*

Abstract
Background: ATM (ataxia-telangiectasia mutated) protein kinase is highly conserved in metazoan, and plays a critical role at DNA damage response, oxidative stress, metabolic stress, immunity, RNA biogenesis etc. Systemic profiling of ATM regulated genes, including protein-coding genes, miRNAs, and long non-coding RNAs, will greatly improve our understanding of ATM functions and its regulation.

Results: 1) differentially expressed protein-coding genes, miRNAs, and long non-coding RNAs in atm mutated flies were identified at physiological condition and after X-ray irradiation. 2) functions of differentially expressed genes in atm mutated flies, regardless of protein-coding genes or non-coding RNAs, are closely related with metabolic process, immune response, DNA damage response or oxidative stress. 3) these phenomena are persistent after irradiation. 4) there is a cross-talk regulation towards miRNAs by ATM, E2f1, and p53 during development and after irradiation. 5) knock-out flies or knock-down flies of most irradiation-induced miRNAs were sensitive to ionizing radiation.

Conclusions: We provide a valuable resource of protein-coding genes, miRNAs, and long non-coding RNAs, for understanding ATM functions and regulations. Our work provides the new evidence of inter-dependence among ATM-E2F1-p53 for the regulation of miRNAs.

Keywords: ATM, X-ray irradiation, miRNA, lncRNA

Introduction
The ataxia-telangiectasia mutated (ATM) protein kinase is a member of the Phosphoinositide-3-Kinase (PI3K)-like Kinase (PIKK) family, and plays a master role to regulate DNA damage response (DDR), especially at double strand breaks (DSBs) repair, to maintain genome integrity [1, 2]. Besides of DNA damage, ATM activation has been shown in connection with other stimuli, such as oxidative stress [3], ATP depletion, mitotic spindle, or metabolic stress, specifically inhibition of glycolysis, which is independent of DNA damage or oxidative stress [1, 2].

†Correspondence: gangcai@ntu.edu.cn; bxl@ntu.edu.cn
1 School of Medicine, Nantong University, Nantong 226001, China
Full list of author information is available at the end of the article

© The Author(s) 2022. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
dependent manner. ATM and ATR (A-T and Rad3-related, another member of PIKK family) phosphorylate more than 700 proteins in response to DNA damage [11]. When ATM is activated by oxidative stress, it can phosphorylate a specific set of proteins that only partially overlap with the phosphorylated proteins in response to the canonical DSBs [12]. In response to replication stress induced by mitomycin C (MMC) and hydroxyurea (HU) treatment, ATM and ATR regulate SUMOylation modification [13]. Thousands of SUMOylation sites and hundreds of phosphorylation sites were identified after treatment, and proteins SUMOylated and phosphorylated are in the overlapping network.

ATM is required for both adaptive and innate immunity, and plays a supportive role in V(D)J-recombination, the core of adaptive immunity [4, 14]. The V(D)J-recombination generates programmed breaks, these breaks are held together in a synapse by the Recombination Activating Genes (RAG) protein. ATM is recruited to RAG-induced DSBs and activated, cooperates with MRN complex, γH2AX, and 53BP1, to stabilize DSBs during V(D)J-recombination, to ensure correct end processing and ligation [14].

Unscheduled transcription and alternative pre-mRNA splicing induced by DNA damage signify great threat to genome integrity. R-loop misregulation can lead to DNA damage, transcription elongation defects and genomic instability. R-loop activates ATM signaling, impedes spliceosome organization, and enhances UV-triggered alternative splicing [15, 16]. ATM and PIKK members influence DNA damage induced transcription through post-translational modification of RNA polymerase II (RNA pol II), transcription elongation factors, and multiple transcription factors, to regulate RNA-dependent DNA damage repair [17, 18]. ATM and ATR phosphorylate components of core spliceosome, promote displacement of spliceosome [1, 18]. Moreover, ATM modulates miRNAs biogenesis by regulating miRNAs transcription, nuclear exporting, processing, and maturation. On the other side, ATM is regulated by miRNAs at post-transcriptional level. ATM-miRNAs work coordinately to regulate DNA damage response and tissue homeostasis [19]. ATM kinase interacts and phosphorylates the KH-type splicing regulatory protein KSRP, a key component in both Drosha and Dicer miRNA-processing complex, enhances KSRP-pri-miRNAs interaction and miRNAs maturation [20].

ATM is required for the maintenance of glucose metabolism. Most A-T patients succumb to metabolic syndromes in early age, show the tendency towards insulin resistance, glucose tolerance, high blood pressure etc. Mutation of atm gene causes loss of redox sensing, increased ROS level, and altered redox balance, which leads to mitochondrial dysregulation and changes of glucose metabolism [1, 4]. A-T patients also develop early-onset cerebellar neurodegeneration, the neurological defect which might be due to loss of redox homeostasis and dysregulation in DNA damage response, however its molecular basis is still not clear [1, 4].

Due to the large number of ATM substrates with different functional features, ATM affects many cellular processes. A systematic genome-wide analysis of genes that may be regulated by ATM will provide a valuable reference for the study of ATM. In this study, we investigated differentially expressed (DE) protein-coding genes, miRNAs, and lncRNAs in atm mutated flies at physiological condition and after ionizing radiation. We found that biological functions of DE genes are diversified, regardless of protein-coding genes or non-coding RNAs, and a lot of DE genes are closely related with metabolic process, immune response, DNA damage response or oxidative stress. These phenomena are persistent even after irradiation treatment. We identified miRNAs regulated by ATM, E2f1 and p53 during development and after irradiation, and provided evidence of a cross-talk regulation among them. We proved that most of irradiation induced miRNAs in atm mutated flies are closely related with DNA damage response.

Materials and methods

Fly genetics

All flies were maintained at 25 °C on standard corn meal unless specified. Fly lines used in this study were: w^{1118} (wild-type, Bloomington Drosophila Stock Center 3605), atm^{agl} (atm) [6, 10], miR-274 KO (Bloomington Drosophila Stock Center 58904); miR-956 KO (Bloomington Drosophila Stock Center 58941) [21], miR-963 sp (Bloomington Drosophila Stock Center 61449); miR-1007 sp (Bloomington Drosophila Stock Center 61490) [22], tubulin-Gal4.

Ionizing radiation

Actively crawling third instar larvae of w^{1118} and atm^{agl} flies were collected and irradiated with 40 Gy, at a dose rate of 340 cGy/min, with X-RAD 320 iX at 320 kV (Precision X-Ray, Inc., North Branford, CT). The irradiated L3 larvae were recovered for 1 h before further experiments.

RNA-seq

Actively crawling third instar larvae of w^{1118} and atm^{agl} flies were collected. There are three replicates in each group with about 30 larvae in each replicate. RNA was purified from irradiated and non-irradiated flies. Total RNA was extracted using Trizol with DNase treatment at the Beijing Genomics Institute (BGI Co., Ltd., China).
The integrity of extracted RNA was assessed by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The mRNA sequencing libraries were constructed with Illumina TruSeq Stranded mRNA Sample Prep Kit (Illumina, Cat. No.RS-122–2101), and paired-end 50 bp sequencing was performed using Illumina HiSeq 2000. The small RNA libraries were constructed with Illumina’s TruSeq Small RNA Sample Prep kit (Illumina, Cat. No. RS-200–0012), and single-end 50 bp sequencing was performed using Illumina HiSeq 2000. All RNA-Seq experiments encompass three biological replicates.

RNA-seq data analysis
For mRNAs and IncRNAs analysis, raw sequencing data quality was assessed with FastQC (v2.0.1) (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and trim_galore (v0.6.6) [23], and aligned to Drosophila melanogaster reference genome (dmel_r6.41_FB2021_04) using STAR (v2.7.9a). Annotated genes were assigned using annotations from Flybase (dmel-all-r6.41.gff) [24] and featureCounts (v2.0.1) [25]. For miRNAs analysis, raw sequencing data quality was assessed using FastQC (v2.0.1). The expression level of annotated miRNAs was quantified by miRDeep2 [26] using the quantifier.pl script with the following parameters: quantifier.pl -m mature.fa -p hairpin.fa -r reads.fa -t dme_index -y filename. Sequence files (mature.fa, hairpin.fa) of annotated miRNAs were retrieved from the miRBase Release 22.1 [27]. Differentially expressed genes (DEGs) were identified by comparison of genes expression in atm-mutated flies with w1118 flies at same experimental condition using R package DESeq2 (version 1.25.12) [28], and a gene is considered differentially expressed when the absolute log2-fold change is ≥ 1 and the adjusted p-value is < 0.05.

Reverse transcription-quantitative PCR
Total RNA was extracted using Trizol and reverse transcribed with Evo M-MLV RT Kit with gDNA Clean for qPCR (Accurate Biology, AG11705) using Oligo dT(18 T) primer 5’ d(TTTTTTTTTTTTTTTTTTTT) 3’. Gene transcripts were quantified by quantitative PCR (qPCR) with SYBR Green Premix Pro Taq HS (Accurate Biology, AG11701) on a Roche LightCycler 96 with comparative Cq method, rp49 was used as a reference gene, and relative expression change was calculated from three independent experiments.

Function enrichment analysis
The Gene Ontology (GO) annotation, KEGG pathway annotation and enrichment analysis were conducted for differentially expressed protein-coding genes and experimentally validated miRNAs targeted genes, based on Gene Ontology database (http://www.geneontology.org/), and KEGG (Kyoto Encyclopedia of Genes and Genomes) (http://www.genome.jp/kegg/) with PANTHER [29] and DAVID (The Database for Annotation, Visualization and Integrated Discovery, https://david.ncifcrf.gov/) [30]. For GO analysis, biological processes (BP) and molecular functions (MF) were applied, GO terms with adjusted p-value <0.05 were considered significant. Significantly enriched biological processes were displayed with R software ggplot2 package and Cytoscape software [31].

Viability assay
Actively crawling third instar larvae were irradiated with the dosage of 0, 10, 20, 30, and 40 Gy, respectively, at a dose rate of 1.3 Gy/min in a X-Ray Biological Irradiator (X-RAD 320iX, Precision X-ray Inc). At least 100 larvae were treated for each genotype at each dosage, and experiments were repeated three times. Survival percentage was calculated as the number of viable adults divided by the number of irradiated third instar larvae. Significant differences between the values under designated experimental conditions were subjected to two-tailed Student’s t-tests. For all tests, a P value less than 0.05 was considered statistically significant.

Results
To explore potential protein-coding genes and non-coding RNAs regulated by ATM in Drosophila, we performed RNA-sequencing (RNA-seq) and small RNA-sequencing (miRNA-seq) on third instar larvae (L3) of wild-type (wt) and atm mutated flies (Fig. 1A). Raw sequencing data of RNA-seq was passed through stringent quality control, and aligned to the Drosophila melanogaster BDGP6 (dmel_r6.41_FB2021_04) using STAR (2.7.9a). In total, 1,025 million reads were collected at an average of 51.25 million reads per sample, range from

(See figure on next page.)
Fig. 1 (See legend on previous page.)
42.3 to 59.7 million reads, and mapped loci range from 68 ~ 96%. We identified 12,584 protein-coding genes with at least 10 reads using featureCounts (v2.0.1) (Table S1), which accounts for 95% of protein-coding genes in Drosophila. Differential expression of the protein-coding gene between the atm and wild-type flies was assessed using DESeq2 (Table S2), and a gene expressed with significant difference (fold change ≥ ±2, adjusted p < 0.05) between the atm and wild-type flies in 3 replicates was regarded as differentially expressed. We identified 462 upregulated and 331 downregulated protein-coding genes in atm mutated flies when compared with wild-type flies (Fig. 1B, Table S3). The expression profile of differentially expressed genes was further verified by RT-qPCR for 10 randomly selected DE genes, including 5 upregulated genes and 5 downregulated genes (Figure S1). We also analyzed the tissue expression characteristics of these differentially expressed genes using tissue expression data from Flyatlas2 (Figure S2) [32].

To investigate the functional annotations of differentially expressed genes, we conducted Gene Ontology enrichment analysis with DAVID. In the GO analysis, the top 10 significantly enriched biological processes were identified in both upregulated and downregulated genes. The highly related biological processes that DE genes involved are cellular process, metabolic process, response to stimulus, developmental process, immune system process, reproduction etc. (Fig. 1C, Table S3). The cellular process and metabolic process are the top 2 biological processes in either upregulated or downregulated DE genes, there are more than 140 DE genes in each of them. And response to stimulus is the number 3 biological process in upregulated DE genes with 111 genes, and number 8 in downregulated DE genes with 43 genes, respectively (Fig. 1C, Table S3). Among DE genes in response to stimulus, they are highly enriched in biological processes such as immune response, stress, bacterial or biotic stimulus etc., and forms several highly connected functional gene clusters (Fig. 1D). Moreover, some DE genes are closely related with DNA damage response or oxidative stress. In upregulated genes, agt is required for DNA dealkylation [33], tie negatively regulate X-ray irradiation induced cell death [34], TotC is induced by oxidation, UV and bacterium treatment [35], timeless is involved into DNA replication checkpoint [36], and corp negatively regulate p53-dependent apoptosis [37]. While in downregulated genes, eya positively regulate DNA repair [36], tfb4 is in response to UV and involved in nucleotide excision repair (NER) pathway [38]. Interestingly, some DE genes play roles in response to light stimulus, including timeless [36], laza [39] and tfb4 [38] in upregulated genes, and glass [40], inaC [41], pthal [42], and rh6 [36] in downregulated genes (Table S3).

As loss of human atm gene causes metabolic syndromes and neurodegeneration, we further investigated their functions in manually curated DE genes. In DE genes related with metabolic process, the top 3 biological processes in upregulated genes are protein metabolic process, cellular aromatic compound metabolic process, and carbohydrate derivative metabolic process. And in downregulated genes, they are cellular aromatic compound metabolic process, protein metabolic process, and nucleic acid metabolic process, respectively (Fig 1E-F, Table S3). Many DE genes are involved in carbohydrate, lipid, amino acid, and RNA metabolic processes, suggesting the critical role that ATM protein kinase plays at metabolism. We also analyzed signaling pathways with KEGG of differentially expressed genes involved, and found that both upregulated and downregulated genes are highly enriched in metabolic pathways (Fig. 1G, Table S3). We further analyzed DE genes related with immunity, and found that some of them play important roles directly in humoral immune response, or innate immune response (Fig. 1H), in which Charon, PGRP-LF, PGRP-SC1b, and PGRP-LC are members in the Imd signaling pathway, and gd, PGRP-SA, and PGRP-SC1b are in the Toll signaling pathway, respectively (Table S3) [43–45]. Charon is an important mediator of PARP-1-dependent transcription in the innate immune pathway [46]. The gd gene is required for the Toll ligand produced by the proteolytic reaction [47]. A previous study performed microarray analysis using adult heads of a temperature sensitive atm allele (atm 8), and identified 117 upregulated and 46 downregulated genes respectively, and found that decreased ATM kinase activity increases expression of innate immune response genes. Moreover, atm knockdown in glial cells resulted in neuronal and glial cell death, decreased mobility and lifespan. When a short hairpin RNA of atm gene was expressed by repo-Gal4 to knock-down atm gene in glial cells, genes expression in 3 ~ 5 days old adult flies were analyzed, there are 246 upregulated genes and 106 downregulated genes respectively, and innate immune response is the most significantly changed biological process [48]. We performed the comparative analysis. In upregulated genes, we identified 33 overlapping genes when compared with repo-ATMi allele, and 27 overlapping genes when compared with atm 8 allele, respectively. While in downregulated genes, there are 12 overlapping genes when compared with repo-ATMi allele, and 3 overlapping genes when compared with atm 8 allele (Fig. 1I), and most of overlapping genes are functional related with immune response.

To study ATM regulated genes in response to DNA damage, we treated the third instar larvae of both wild-type and atm flies with irradiation, and identified 129 upregulated genes and 61 downregulated genes (Fig. 1),
Table S4). We also analyzed the tissue expression characteristics of differentially expressed genes after irradiation (Figure S3). Among DE genes after irradiation, many of them are related with biological processes such as cellular process, metabolic process, developmental process, reproduction etc., although the number of differentially expressed genes was significantly reduced when compared with that of no irradiation treatment, which strengthen the previous suggestion that the fine tune of metabolic demands by communicating with surrounding milieu in response to stress is required in multicellular organisms [49].

**ATM regulates miRNAs biogenesis at both physiological condition and after ionizing radiation**

As ATM plays an important role at regulating miRNAs biogenesis, we further investigated differentially expressed miRNAs in *atm* flies at physiological condition (NP) and in response to ionizing radiation (IR). Total RNA was prepared and small RNA-seq was performed. Raw sequencing data was processed with cutadapt to remove adapter sequence and generate the clean miRNA data. The miRNA reads were ranged from 38.4 to 54.6 million (Table S5), and mapped to genome with miRBase Release 22.1 using mirdeep2 (2.0.1.3). In total, we identified 281 annotated mature miRNAs with at least 10 reads (Table S5). The differential expression of miRNAs was assessed using DESeq2, and a miRNA gene expressed with significant difference (fold change ≥ ±2, adjusted p < 0.05) between the *atm* and wild-type flies in 3 replicates was regarded as differentially expressed.

Compared with wild-type flies, we identified 6 upregulated miRNAs, including miR-274-5p, miR-956-5p, miR-956-3p, miR-986-5p, and miR-1007-5p, and 11 downregulated miRNAs in *atm* flies, including let-7-5p, miR-210-5p, miR-310-5p, miR-311-5p, miR-311-3p, miR-963-3p, miR-981-3p, miR-982-3p, miR-983-5p, miR-984-5p, and miR-4944-5p (Fig. 2A, Table S6). In upregulated miRNAs with annotated functions, *miR-274-5p* coordinates nervous and vascular development in glia, regulates circadian behavior in astrocytes, and plays a role in response to hypoxia [50, 51]; *miR-956-5p* is related with muscular dystrophy and has the pro-virulence function [52, 53]; *miR-980-5p* regulates axon guidance and is related with muscular dystrophy, suppresses memory in adult brain, and is downregulated upon starvation and metabolic stress [52, 54, 55]. In downregulated miRNAs with annotated functions, *let-7-5p* forms a cluster with *miR-100* and *miR-125*, they play critical role during metamorphosis [56] and have diversified functions; *miR-210-5p* modulates circadian rhythms, locomotion, and lipid metabolism, and prevents neurodegeneration in retina [57–59]; *miR-310-5p*, *miR-311-5p* and *miR-311-3p* in the *miR-310/313* cluster transduce nutritional signals as a metabolic regulator, are required for normal synaptic transmission, and involved into Toll pathway mediated immune response by regulating expression of the antimicrobial peptide Drosomycin [60–62]; *miR-981-3p* negatively regulates anti-bacterial defense by reducing diptericin in IMD pathway [62]. While *miR-986-5p* and *miR-1007-5p* in upregulated genes, and *miR-963-3p*, *miR-982-3p*, *miR-983-5p*, *miR-984-5p*, and *miR-4944-5p* in downregulated genes have no annotated functions, their functions might be closely connected with ATM, which needs further investigation.

Furthermore, we identified 5 upregulated miRNAs, including *miR-274-5p*, *miR-956-5p*, *miR-956-3p*, *miR-986-5p*, and *miR-1007-5p*, and 2 downregulated miRNAs, including *miR-963-3p* and *miR-4944-5p*, in *atm* flies after irradiation (Fig. 2B, Table S7). We treated miRNA knock-out or knock-down flies with X-ray irradiation, looked at their survival rates to investigate whether they are sensitive to ionizing radiation. Our previous result has shown that *miR-956 KO* and *miR-986 KO* flies are highly sensitive to irradiation [63], while *miR-274 KO* flies are not sensitive to irradiation, indicating that although *miR-274-5p* expression was increased after irradiation, it may not play a role at DNA damage response. We used *tubulin-Gal4* to drive ubiquitous expression of *miR-963* and *miR-1007*, and found that *tubulin-miR-1007i* (tub-miR-1007i) flies with *miR-1007-5p* knock-down are sensitive to irradiation, while *tubulin-miR-963i* (tub-miR-963i) flies with *miR-963-3p* knock-down are not sensitive to irradiation (Fig. 2C). As miRNAs play a role to fine tune genes expression and activities, residual expression level of *miR-963-3p* may influence the DNA damage sensitivity, a knock-out mutant of *miR-963-3p* is required to test whether it plays a role at DNA damage response. We did not test *miR-4944-5p* as no allele is available.

We performed comparison analysis with DE miRNAs in *p53* or *e2f1* mutated flies [63]. At physiological condition, *miR-274-5p* was upregulated in *atm*, *p53* and *e2f1* mutated flies, *miR-963-3p* was downregulated in *atm*, *p53* and *e2f1* mutated flies, *miR-986-5p* was upregulated in *atm* and *e2f1* mutated flies, and *miR-310-5p* was downregulated in *atm* and *p53* mutated flies. After irradiation, *miR-963-3p* was downregulated in *atm*, *p53*, and *e2f1* mutated flies, *miR-274-5p* and *miR-986-5p* were upregulated in *atm* and *e2f1* mutated flies (Fig. 2D), suggesting a cross-talk regulation to miRNAs among ATM, E2f1 and p53 during development and after irradiation.

**Differentially expressed IncRNAs in atm mutated flies**

We further investigated differentially expressed IncRNAs in *atm* flies at physiological condition and in response to ionizing radiation. RNA preparation, data processing,
**Fig. 2** Differentially expressed miRNAs in atm mutated flies at physiological condition and after ionizing radiation. 

**A** Heatmap of miRNAs differentially expressed between atm mutated flies and wild-type flies. 

**B** Heatmap of miRNAs differentially expressed between atm mutated flies and wild-type flies after ionizing radiation. 

**C** Sensitivity of knock-out or knock-down flies of DE miRNAs to ionizing radiation. 

**D** Relative expression of DE miRNAs in atm, p53 and e2f1 mutated flies. *p ≤ 0.05; n.s., no significance. Error bars indicate SEM.
and identification of differentially expressed IncRNAs were performed as protein-coding genes. We identified 1128 IncRNAs with at least 10 reads (Table S8). Compared with wild-type flies, we identified 45 upregulated IncRNAs and 32 downregulated IncRNAs in atm flies at physiological condition (Fig. 3A, Table S9), and 15 upregulated IncRNAs and 9 downregulated IncRNAs in atm flies after irradiation (Fig. 3B, Table S10). Until now, very few IncRNAs in Drosophila have been studied, differentially expressed IncRNAs in atm mutated flies might play diversified roles as ATM kinase does. Three differentially expressed IncRNAs, including CR31781, which is upregulated in atm flies at physiological condition, and CR43282 and CR43356, which are downregulated in atm flies at physiological condition, have been studied in systemic analysis. CR31781 is involved in muscle lateral inhibition [64], and CR43282 and CR43356 knock-out flies have reduced male fertility [65]. These three functional DE IncRNAs play different functions in Drosophila, such as in development and production, which is consistent with that of differentially expressed protein-coding genes and microRNAs.

Discussion

The ATM protein kinase plays an important role and has diversified functions during development and in response to stress. Study of ATM regulated genes had been focused on post-translational modifications such as phosphorylation, somoylation etc. In this study, we systemically profiled differentially expressed genes in Drosophila, including protein-coding genes, miRNAs and IncRNAs, in atm mutated flies at physiological condition and after irradiation. We identified hundreds of differentially expressed protein-coding genes, and multiple miRNAs and IncRNAs. For those protein-coding genes and miRNAs with annotated functions, their functions are diversified and complex, span metabolism, immune response, multicellular organismal process, response to stimulus etc., which is consistent with our current understanding of the complex functions of ATM kinase.

The DNA damage response is highly conserved from invertebrates to mammals. Activation of systemic response is executed in response to DNA damage, including metabolic change, immune response etc., which plays very important role for the physiological adjustments for remodeling or regeneration of tissues and organs. Due to its simpler yet conserved genetics and physiology, Drosophila is of particular importance to investigate local and systemic interactions in response to stimulus. Moreover, compared with previous studies that showing proteins phosphorylated by ATM, our study provides a new angle of view to understand ATM functions and its downstream targets. Furthermore, by doing comparation analysis with our previous work studying miRNAs regulated by p53 and E2F1 [63], we identified miRNAs differentially expressed in atm mutated flies, p53 mutated flies or e2f1 mutated flies, and provide new evidence of inter-dependent regulation of miRNAs among ATM-E2F1-p53.

Conclusions

Differentially expressed genes, including protein-coding genes and non-coding RNAs, in atm mutated flies have diversified functions and are highly
related with metabolism, immune response, multicellular organismal process, response to stimulus etc. These phenomena are persistent after irradiation. There is a cross-talk regulation towards miRNAs by ATM, E2fl and p53 during development and after irradiation.

Abbreviations
ATM: Ataxia-telangiectasia mutated; DDR: DNA damage response; DE: Differentially expressed; DEGs: Differentially expressed genes; DSB: Double strand breaks; GO: Gene Ontology; IR: Ionizing radiation; KEGG: Kyoto Encyclopedia of Genes and Genomes; L3: Third instar larvae; NP: Physiological condition; PIKK: Phosphoinositide-3-Kinase (PI3K)-like Kinase; RNA-seq: RNA-sequencing.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s41065-022-00254-9.

Additional file 1: Figure S1. RT-qPCR validation of DE protein-coding genes identified by RNA-seq. Validation of 5 upregulated and 5 downregulated protein-coding genes in atm mutated flies. Error bars indicate SEM.

Additional file 2: Figure S2. Tissue expression of differentially expressed protein-coding genes in atm mutated flies at physiological condition. A Heatmap of differentially expressed genes expressed in larval tissues. B Heatmap of differentially expressed genes expressed in adult female tissues. C Heatmap of differentially expressed genes expressed in adult male tissues.

Additional file 3: Figure S3. Tissue expression of differentially expressed protein-coding genes in atm mutated flies after ionizing radiation. A Heatmap of differentially expressed genes expressed in larval tissues. B Heatmap of differentially expressed genes expressed in adult female tissues. C Heatmap of differentially expressed genes expressed in adult male tissues.

Additional file 4: Table S1. mRNA counts.

Additional file 5: Table S2. Protein-coding genes sequenced.

Additional file 6: Table S3. Differentially expressed protein-coding genes in atm mutated flies.

Additional file 7: Table S4. Differentially expressed protein-coding genes in atm mutated flies after X-ray irradiation.

Additional file 8: Table S5. miRNA counts.

Additional file 9: Table S6. Differentially expressed lncRNAs in atm mutated flies.

Additional file 10: Table S7. Differentially expressed lncRNAs in atm mutated flies after X-ray irradiation.

Additional file 11: Table S8. Differentially expressed protein-coding genes in atm mutated flies at physiological condition.

Additional file 12: Table S9. Differentially expressed lncRNAs in atm mutated flies.

Acknowledgements
We thank the Bloomington Stock Center for fly stocks, members of the Bi laboratory for advices and discussions.

Authors' contributions
X.B. conceived project and designed experiments; JL performed bioinformatic analysis; T.J., L.R., Z.Z., and R.Z. performed experiments; J.L., G.X., and X.B. analyzed data, and X.B. wrote the manuscript. The author(s) read and approved the final manuscript.

Authors' information
Not applicable.

Funding
This work was supported by grants from NationalNatural Science Foundation of China Grant No. 31771437 and 31970605 to X.B., 31900484 to G.X.

Availability of data and materials
The raw sequencing data generated in this study has been submitted to the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/) under accession number PRJNA809845. The custom code used is available at https://github.com/Liu970101/atm.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors agreed for this publication.

Competing interests
The authors declare that they have no conflict of interest.

Author details
1School of Medicine, Nantong University, Nantong 226001, China. 2College of Basic Medical Medicine, Dalian Medical University, Dalian 116044, China.

Received: 6 June 2022 Accepted: 6 October 2022
Published online: 21 October 2022

References
1. Lee JH, Paull TT. Cellular functions of the protein kinase ATM and their relevance to human disease. Nat Rev Mol Cell Biol. 2021;22(12):796–814.
2. Paull TT. Mechanisms of ATM Activation. Annu Rev Biochem. 2015;84:711–38.
3. Guo Z, Kozloff S, Lavin MF, Person MD, Paull TT. ATM activation by oxidative stress. Science. 2010;330(6003):517–21.
4. Zaki-Dizaji M, Akrami SM, Abolhassani H, Rezaei N, Aghamohammadi A. Ataxia telangiectasia syndrome: moonlighting ATM. Expert Rev Clin Immunol. 2017;13(12):1155–72.
5. Daniel JA, Pellegri M, Lee BS, Guo Z, Filsuf D, Belkina NV, You Z, Paull TT, Sleckman BP, Feigenbaum L, et al. Loss of ATM kinase activity leads to embryonic lethality in mice. J Cell Biol. 2012;198(3):295–304.
6. Bi X, Wei SC, Rong YS. Telomere protection without a telomerase; the role of ATM and Mre11 in Drosophila telomere maintenance. Curr Biol. 2004;14(15):1341–7.
7. Silva E, Tiong S, Pedersen M, Homola E, Royou A, Fasulo B, Siracoo G, Campbell SD. ATM is required for telomere maintenance and chromosome stability during Drosophila development. Curr Biol. 2004;14(15):1341–7.
8. Song YH, Mirey G, Betson M, Haber DA, Settleman J. The Drosophila ATM ortholog, dATM, mediates the response to ionizing radiation and to spontaneous DNA damage during development. Curr Biol. 2004;14(15):1354–9.
9. Bi X, Gong M, Srikanth D, Rong YS. Drosophila ATM and Mre11 are essential for the G2/M checkpoint induced by low-dose irradiation. Genetics. 2005;171(2):845–7.
10. Gong M, Bi X, Rong YS. Targeted mutagenesis of Drosophila atm and mre11 genes. Drosoph Inf Serv. 2005;84:79–83.
11. Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER 3rd, Hurov KE, Luo J, Bakalarski CE, Zhao Z, Solimini N, Leder P, et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science. 2007;316(5828):1160–6.
12. Yan S, Sorell M, Berman Z. Functional interplay between ATM/ATR-mediated DNA damage response and DNA repair pathways in oxidative stress. Cell Mol Life Sci. 2014;71(20):3951–67.
13. Munk S, Siguretschoon JO, Xiao Z, Batth TS, Franciosa G, von Stechow L, Lopez-Contreras AJ, Vertegaal ACO, Olsen JV. Proteomics Reveals Global Regulation of Protein SUMOylation by ATM and ATR Kinases during Replication Stress. Cell Rep. 2017;21(2):546–58.
14. Wetering T, Takada S, Weemaes CMR, van Schouwenburg PA, van der Burg M. ATM: Translating the DNA Damage Response to Adaptive Immunity. Trends Immunol. 2021;42(4):350–65.
15. Niehs C, Luke B. Regulatory R-loops as facilitators of gene expression and genome stability. Nat Rev Mol Cell Biol. 2020;21(3):167–78.
16. Tresini M, Warmerdam DO, Kolovos P, Snijder L, Vouwe MG, Demmers JA, van Lier J, Grosveld F, Medema RH, Hoeijmakers JH, et al. The core spliceosome as target and effector of non-canonical ATM signalling. Nature. 2015;523(7558):53–8.
17. Burger K, Ketley RF, Gullerova M. Beyond the Trinity of ATM, ATR, and DNA-PK: Multiple Kinases Shape the DNA Damage Response in Concert With RNA Metabolism. Front Mol Biosci. 2019;6:61.
18. Angarola BL, Anczukow O. Splicing alterations in healthy aging and disease. Wiley Interdiscip Rev RNA. 2021;12(4):e1643.
19. Rezaeian AH, Khanbabaei H, Calin GA. Therapeutic Potential of the O6-alkylguanine-DNA alkyltransferase cDNA. Nucleic Acids Res. 1999;27(8):1795–801.
20. Zhang X, Wan G, Berger FG, He X, Lu X. The ATM kinase induces microRNA biogenesis in the DNA damage response. Mol Cell. 2011;41(1):371–83.
21. Chen YY, Song S, Wang R, Verma P, Kugler JM, Buescher M, Rouam S, Cohen SM. Systematic study of Drosophila microRNA functions using a collection of targeted knockout mutations. Dev Cell. 2013;31(6):784–800.
22. Fulga TA, McNeill EM, Binari R, Yelick J, Blanche A, Booker M, Steinkraus BR, Schnall-Levin M, Zhao Y, DeLuca T, et al. A transgenic resource for functional annotation of all Drosophila microRNAs. Nat Commun. 2015;6:7279.
23. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose sequencing to function aligner. Bioinformatics. 2013;29(1):15–21.
24. Thurmond J, Goodman JL, Streltes VB, Attrill H, Gramates LS, Marygold SJ, Matthews BB, Millburn G, Antonazzo G, Trovisco V, et al. FlyBase 2.0: the Next generation of FlyBase. Nucleic Acids Res. 2013;41(1):D755–69.
25. Love MI, Huber W, Anders S. Moderated estimation of fold change and effect size in RNA sequencing (RNA-seq). Ann Appl Stat. 2014;8(2):988–1004.
26. Dong H, Han JH, Lee SH, Tan YQ, E K LeMeys, Hashimoto C. Gastrulation defective is a serine protease involved in activating the receptor toll to polarize the Drosophila embryo. Proc Natl Acad Sci USA. 2000;97(16):9093–7.
27. Petersen AJ, Rimkus SA, Wassarman DA. ATM kinase inhibition in glial cells activates the innate immune response and causes neurodegeneration in Drosophila. Proc Natl Acad Sci U S A. 2012;109(1):E656–664.
28. Chatzidoukaki O, Goulielmaki E, Schumacher B, Garinis GA. DNA Damage Response and Metabolic Reprogramming in Health and Disease. Trends Endocrinol. 2020;31(6):779–91.
29. Lyu J, Chen Y, Yang W, Guo T, Xu X, Xi Y, Yang X, Ge W. The conserved miR-240-5p regulates autophagy during Drosophila development. Cell Death Differ. 2015;22(1):764–79.
30. Cicek IO, Karaca S, Branikatsch M, Eaton S, Urlaub H, Shcherbata HR. The conserved microRNA miR-210 regulates insulin signaling and neuronal polarity during Drosophila development. Cell Death Differ. 2015;22(1):764–79.
31. Mukherjee S, Koh HH, Seow CY, Tan WS, Chen SC, Sheng W, Gao L, Wang Y, Chen D, Wang S, et al. miR-210 promotes Drosophila neurodevelopment. Sci Adv. 2015;1(6):e1500643.
32. Angarola BL, Anczukow O. Splicing alterations in healthy aging and disease. Wiley Interdiscip Rev RNA. 2021;12(4):e1643.
61. Tsurudome K, Tsang K, Liao EH, Ball R, Penney J, Yang JS, Elazzouzi F, He T, Chishti A, Lnenicka G, et al. The Drosophila miR-310 cluster negatively regulates synaptic strength at the neuromuscular junction. Neuron. 2010;68(5):679–93.
62. Li S, Shen L, Sun L, Xu J, Jin P, Shen L, Ma F. Small RNA-Seq analysis reveals microRNA-regulation of the Imd pathway during Escherichia coli infection in Drosophila. Dev Comp Immunol. 2017;70:80–7.
63. Li D, Ge Y, Zhao Z, Zhu R, Wang X, Bi X. Distinct and Coordinated Regulation of Small Non-coding RNAs by E2F1 and p53 During Drosophila Development and in Response to DNA Damage. Front Cell Dev Biol. 2021;9:695311.
64. Mummery-Widmer JL, Yamazaki M, Stoege T, Novatchkova M, Bhalerao S, Chen D, Dietzl G, Dickson BJ, Knoblisch JA. Genome-wide analysis of Notch signalling in Drosophila by transgenic RNAi. Nature. 2009;458(7241):987–92.
65. Wen K, Yang L, Xiong T, Di C, Ma D, Wu M, Xue Z, Zhang X, Long L, Zhang W, et al. Critical roles of long noncoding RNAs in Drosophila spermatogenesis. Genome Res. 2016;26(9):1233–44.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.