Biomaterials 32 (2011) 3807–3814

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

MicroRNAs as participants in cytotoxicity of CdTe quantum dots in NIH/3T3 cells

Shuchun Li, Yong Wang, Haitao Wang, Yunfei Bai, Gaofeng Liang, Yuanyuan Wang, Ningping Huang, Zhongdang Xiao*

State Key Laboratory of Bioelectronics, School of Biological Science & Medical Engineering, Southeast University, #2 Si Pai Lou, Nanjing 210096, PR China

ARTICLE INFO

Article history:
Received 6 January 2011
Accepted 30 January 2011
Available online 3 March 2011

Keywords:
MicroRNA
CdTe QDs
Cytotoxicity
p53
miRNA biogenesis

ABSTRACT

Epigenetic aspects of the cytotoxicity of CdTe quantum dots (QDs) recently have attracted more attention for their ability to reprogram gene expression after initial signals have been removed. And the involvement of epigenetic mechanisms in microRNA (miRNA) biogenesis suggests that miRNAs act as participants in the cytotoxicity of CdTe QDs. According to the results of SOLiD sequencing, the expression patterns of miRNAs are widely affected after CdTe QD exposure, resulting in the apoptosis-like cell death. Compared with 86 miRNAs with down-regulated expression, the expression levels of 121 miRNAs are up-regulated by CdTe QD treatment. The Z-test is used to find out miRNAs with significantly regulated expression, and the results indicate that the expression levels of 16 and 35 miRNAs are down- and up-regulated, respectively. And the expression levels of some significantly regulated miRNAs have time- and dose-dependent tendencies, which are similar to cell survival ratios affected by CdTe QDs. The fluctuations of miRNA expression start from the transcription of pri-miRNA, and are strengthened by the processing of pri-miRNA to pre-miRNA. As a regulator in miRNA biogenesis, *p53* is involved in the transcription and processing of pri-miRNA. With no significant changes in the mRNA levels of *p53*, the increase in overall *p53* protein levels and its post-translational modification by phosphorylation at Ser-15 are induced by CdTe QD treatment. Therefore, the differential expression of miRNAs are induced by CdTe QDs at the processing of miRNA biogenesis, which is an adaptive process of cells to external stimuli.

1. Introduction

The wide applications of CdTe QDs in medical imaging have aroused global concern regarding their influences on biological systems, resulting in a demand for parallel risk assessment [1,2]. Based on the published studies on biocompatibility of CdTe QDs *in vitro* [3–6], the cytotoxicity of CdTe QDs can be divided into three aspects, non-genotoxic, genotoxic [7] and epigenetic agents [8,9]. Among them, the non-genotoxic agents have been widely analyzed by common cellular methods (MTT, WST-1 and CCK-8). But the genotoxic and epigenetic agents have attracted more attention recently for their important roles in the cytotoxicity of CdTe QDs [8–12]. The genotoxic agents damage DNAs, which then results in loss of DNA integrity, mutagenesis and chromosomal aberrations [10,11]. In contrast, the epigenetic agents modify the expression of genetic information without altering the primary DNA sequences [8,9], which might lead to a long-term reprogramming of gene expression after the initial signals have been removed [13]. Therefore, it is more important to consider the epigenetic effects of CdTe QDs to fully evaluate their cytotoxicity.

miRNAs are small non-coding RNAs that repress the translation of complementary mRNAs and function as key controllers in a myriad of cellular processes. Thus, the expression of miRNAs are under tight regulation through many mechanisms. Among those regulation mechanisms, epigenetic mechanisms, such as promoter methylation or histone acetylation, can modulate the microRNA expression, and an aberrant regulation at this level is found in different diseases [14]. Meanwhile, the differential miRNA expression may be consequences of epigenetic modification. Among those mostly researched epigenetic modification, the histone modification has been reported to be performed by CdTe QD exposure [8,9], which suggests the possibility of involving miRNAs in the cytotoxicity of CdTe QDs. In addition, according to the non-genotoxic assessment, the apoptosis-like cell death are commonly induced by CdTe QDs in many cell lines [7]. And the expression of some proteins are regulated at the post-transcriptional level with no significant changes in the expression levels of mRNAs [9]. The phenomenon suggests that miRNAs play a role in the cytotoxicity of CdTe QDs at the post-transcriptional level.

The dysregulated expression of miRNAs is not only an adaptive process to extracellular stimuli, but also a consequence of the

* Corresponding author. Tel./fax: +86 25 83790820.
E-mail address: zdxiao@seu.edu.cn (Z. Xiao).

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.01.074

disparity between miRNA biogenesis and miRNA degradation. Since miRNA biogenesis plays the most important role in dysregulated expression of a miRNA, more attention is paid to explore mechanisms involved in miRNA biogenesis. Although major process has been made in understanding the basic mechanisms of miRNA biogenesis, the mechanisms which govern the regulation of miRNA biogenesis and activity are still not completely understood. Following the transcription by RNA polymerase II, mature miRNAs are generated through a series of coordinated processing events mediated by large protein complexes. And each step of the general biogenesis pathway is differentially regulated to allow the exquisite control of miRNA expression. The regulation degree of each processing step in miRNA biogenesis can be determined by measuring the expression levels of miRNA precursors, pri-miRNAs and pre-miRNAs [15]. Thus, the influences of CdTe QD exposure on the processing of miRNA biogenesis were investigated in this paper.

2. Methods

2.1. Preparation of CdTe QDs

High quality oil-soluble CdTe quantum dots were synthesized in liquid paraffin wax according to Xing Bin et al. [16]. Then, water-soluble CdTe QDs were prepared with short-chain thioglycolic acid (TGA) as described by Wang Yong et al. [17]. Transmission electron microscopy (TEM) images were taken with a Tecnai 20 microscope [10]. TEM samples were prepared by dropping the freshly prepared solution onto a 300-mesh carbon-coated copper grid.

2.2. Cell culture and CdTe QD treatment

NIH/3T3 cells (SIBS, China) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Canada) containing 10% fetal bovine serum (FBS) (Gibco, Canada) and 1% penicillin–streptomycin (Gibco, Canada) at 37 °C in a 5% CO_2 incubator (Hera Cell 150, Thermo).

Cells were seeded at an initial density of 2×10^4 cells ml^{-1} in 35 mm culture dishes. To extract total RNAs from cells, 24 h after seeding, cells were incubated with CdTe QDs (Fig. 1) at terminal concentrations of 15 μg/ml, 30 μg/ml and 45 μg/ml for 12 h and 24 h, respectively. All treatments were done in triplicate in three independent experiments.

2.3. Cell viability determined by WST-1 assay

After the incubation with CdTe QDs, the cell viability of NIH/3T3 cells was determined by the WST-1 [6] according to the manufacturer's instructions. For this procedure, cells were seeded in 96-well plates and cultured in DMEM for 24 h. Cells were then stimulated with CdTe QDs at 0, 15, 30, and 45 μg/ml for 12 h and 24 h, respectively. Assays were performed by adding WST-1 directly to culture wells and incubating them for 120 min at 37 °C. The plates were then read by a scanning multi-well spectrophotometer by measuring the absorbance of the dye with a wavelength of 450 nm. All measurements were taken in triplicate from three independent experiments.

2.4. Small RNA Extraction and SOLiD sequencing

After the 24 h incubation with 30 μg/ml CdTe QDs, cells were harvested with trypsin. Small RNAs were extracted with the mirVana™ miRNA isolation kit (Ambion) according to the manufacturer's protocols.

The SOLiD™ Small RNA Expression Kit (P/N 4397682) enabled the conversion of small RNAs in a sample into a double-stranded cDNA library that was compatible with the Applied Biosystems SOLiD™ System for the next generation high-throughput sequencing. The results of SOLiD developed by ABI could be in the form of nucleotide sequences and their coverage. By consequently comparing those sequences with the Genbank (www.ncbi.nlm.nih.gov/Genbank) and the miRbase (http://www.mirbase.org), registered miRNAs could be determined from all detected sequences.

2.5. Z-test

For the drawbacks of fold-change analysis, the Z-test was chosen to analyze the expression patterns of miRNAs. The method used was to look at the number of copies of a specific miRNA in a cell as a fraction or a proportion of the total number of miRNA molecules in that cell [18,19].

$$p = \frac{n_{\text{specific miRNA/cell}}}{N_{\text{total miRNA/cell}}} = \frac{n_{\text{specific miRNA}}}{N_{\text{total miRNA}}} \quad (1)$$

So the Z-value of a specific miRNA could be calculated as follows:

$$Z = \frac{p_{\text{test}} - p_{\text{control}}}{\sqrt{p_0(1-p_0)/N_{\text{test}} + p_0(1-p_0)/N_{\text{control}}}} \quad (2)$$

There p_0 was calculated as $p_0 = (n_{\text{test}} + n_{\text{control}})/(N_{\text{test}} + N_{\text{control}})$, which was the estimate of the proportion if the null hypothesis was true. If the Z-score >1.96, it meant that the miRNA expression was significantly up-regulated. And if the Z-score <-1.96, it meant that the miRNA expression was significantly down-regulated.

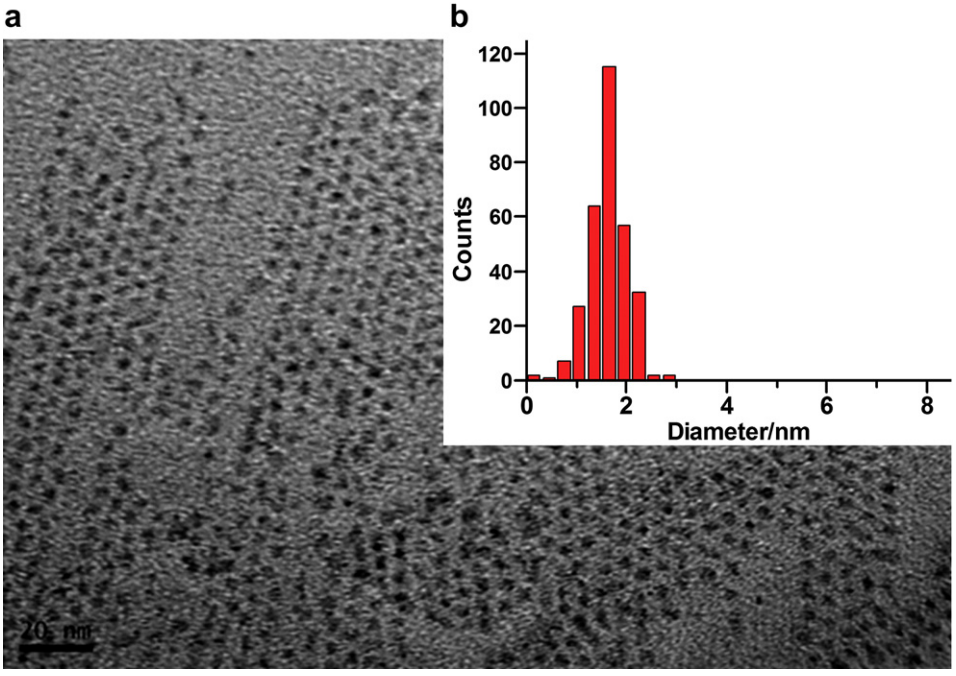

Fig. 1. TEM micrographs (a) and the diameter distribution (b) of CdTe QDs used in this paper.

2.6. Reverse-transcription and qRT-PCR

Total RNAs were extracted using the Trizol (Invitrogen, UK) according to the manufacturer's protocols. The concentration of a RNA sample was performed by measuring the absorbance at 260 nm (A_{260}) in a spectrophotomometer, and RNA purity was indicated with the ratio of A_{260} to A_{280}.

For mature miRNAs and their precursors, the protocols of reverse-transcription were the same except for reverse-transcription primers. Mature miRNAs were reverse-transcribed with the loop primers, while the primary miRNAs and the precursor miRNAs were reverse-transcribed with the reward primers applied in PCR (Supplement Table). The cDNA synthesis was performed with a 2720 Thermal Cycler (Applied Biosystems) in a 20 μl reaction volume containing 1 μg total RNA, 0.5 μM reverse-transcript primers, 50 mM Tris–HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 20 units ribonuclease inhibitor and 100 U PrimerScript™ reverse transcriptase (Takara, China). After incubation at 42 °C for 1 h, reverse transcriptase was inactivated at 85 °C for 5 min and the cDNAs were stored at −20 °C or immediately used for real-time PCR.

After the cDNA synthesis, the relative expression levels were determined by real-time PCR in an ABI 7500 real-time PCR system with Evagreen (Biotium, USA). All reactions were performed in triplicate with the same cDNA samples and, hence, represented technical replicates. The Takara Tag™ Hot Start version (Takara, China) was used according to the manufacturers suggestions. Briefly, 1 μl cDNA was used for a 20 μl PCR reaction containing 0.5 U Tag™ Hot Start PCR enzyme, 0.2 mM dNTPs, 0.4 μM forward primer, 0.4 μM reward primer, 1 μl Evagreen, 0.4 μl ROX (Takara, China). PCR was achieved with a 10 min activation and denaturation step at 95 °C, followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. The baselines and the thresholds for Ct calculation were set automatically with the ABI Prism 7500 SDS software version 1.1, or set manually whenever necessary.

2.7. Western blotting

After the incubation with changing doses of CdTe QDs for 12 h and 24 h, cells were harvested and washed twice with PBS. The total proteins were extracted with the Complete Lysis-M [6]. 70 μg total protein extracts were loaded onto a gradient SDS-PAGE gel and transferred to a nitrocellulose membrane. The membrane was blocked with 5% milk for 1 h and incubated with the primary antibody in the blocking solution for 1 h. After twice washes with 0.1% Tween-20 and TBS, the membrane was incubated with the secondary anti-rabbit antibody diluted 1:4000 in 5% milk, 0.5% Tween-20 and TBS for 1 h. After thrice washes with 0.1% Tween-20 and TBS, the membrane was exposed to film, and a single band was observed. The primary antibodies used were anti-*p53* (sc-6243, 1:500) (Santa Cruz Biotechnology), anti-actin (sc-1616, 1:1000) (Santa Cruz Biotechnology) and antiphospho-*p53* (Ser15–9284, 1:1000) (Cell Signaling Technology).

2.8. Statistical analysis

The relative expression ratios were analyzed with $2^{-\Delta\Delta Ct}$ [20], and the $-\Delta\Delta Ct$ was calculated as follows.

$$-\Delta\Delta Ct = -[(Ct_{RNA} - Ct_{U6})_{test} - (Ct_{RNA} - Ct_{U6})_{control}] \quad (3)$$

in which, RNA represented miRNA, pri-miRNA and pre-miRNA. In addition, the Pearson correlation was performed with SPSS 17.

3. Results and discussions

3.1. Non-genotoxic effects of CdTe QDs

To illustrate the effects of changing concentrations of CdTe QDs on cell survival and morphology, several assays were used to study the influences of CdTe QDs on cell proliferation, growth and metabolic activity. After being exposed to CdTe QDs, cells were seen to be round by live cell imaging with an optical microscope (Fig. 2a and b). A dose-dependent decrease in cell viability was observed, with a higher concentration of QDs leading to more round cells. In addition, a time-dependent decrease in cell viability was also observed, with a longer CdTe QD exposure time leading to lower cell viability.

Subsequently, the WST-1 analysis was performed to determine the influences of CdTe QD exposure on metabolic activity of NIH/3T3 cells. The metabolic activity kept decreasing with the increasing exposure doses of CdTe QDs. The cellular survival ratios, calculated according to the formula described by the Ref. [21], decreased in a dose- and time-dependent manner (Fig.2c), which confirmed the optical microscope studies of cellular proliferation.

Even though CdTe QDs are capped with TGA, free Cd^{2+} are still present in the particle suspension or released from the CdTe core within cells [5,7,22,23]. And the Cd^{2+} are responsible for the cytotoxicity of CdTe QDs via several pathways including interference of DNA repair, substitution for physiologic Zn, and induction of reactive oxygen species (ROS). Recently, the cytotoxicity of CdTe QDs is reported to be based on the concentration of total QDs ingested by cells except for the released Cd^{2+} from cells [5]. A significant dose-dependent increase in the $[\text{total } Cd^{2+}]_{intracellular}$ is observed with a higher concentration of CdTe QDs leading to higher intracellular

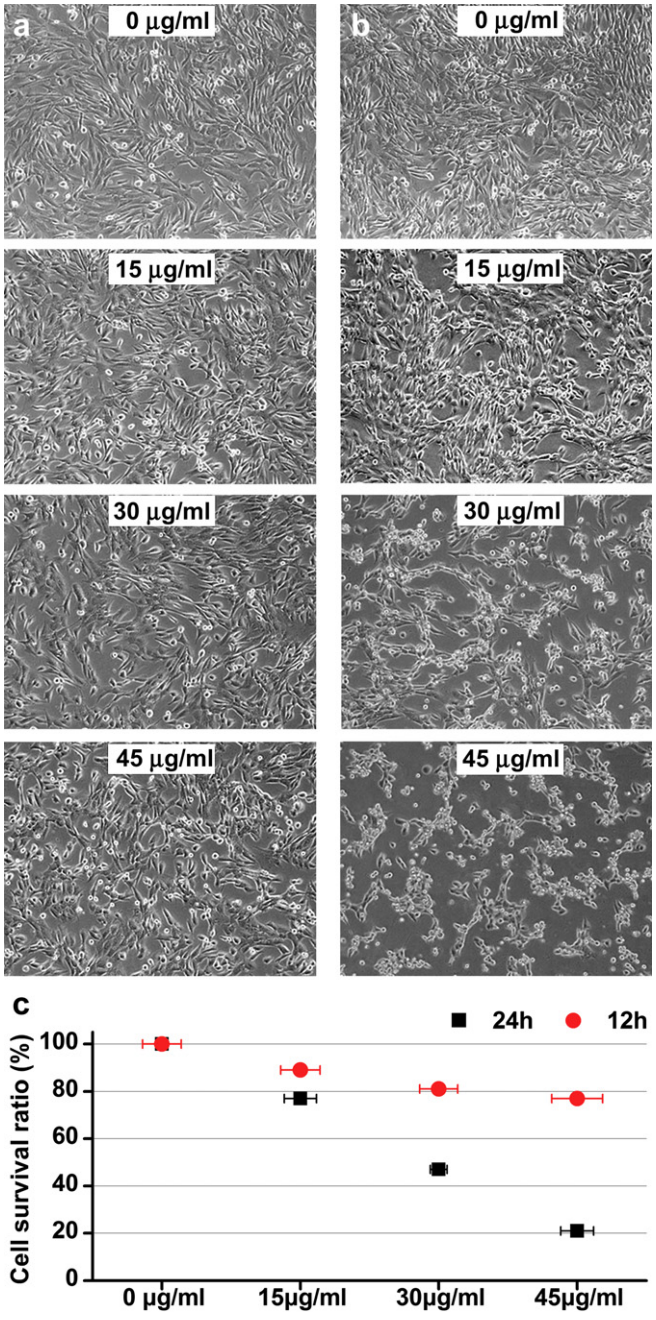

Fig. 2. Non-genotoxic cytotoxicity of CdTe QDs. Live cell imaging with electron microscope was performed to detect the effects of CdTe QDs on cellular morphology for 12 h (a) and 24 h (b) exposure. Apoptosis-like cell death was caused under effort of CdTe QDs. WST-1 analysis was performed to detect the influence of CdTe QD exposure on metabolic activity in NIH/3T3 cells (c). WST-1 data indicated that metabolic activity was decreased for the increasing exposure doses of CdTe QDs. And cellular survival ratio was decreased in a dose-dependent manner.

total Cd^{2+}. Thus, the cytotoxicity of CdTe QDs has dose- and time-dependent tendencies, which is confirmed by our research and others [24].

3.2. miRNAs as participants in cytotoxicity of QDs

miRNAs, repressors of gene expression at the post-transcription level, are widely involved in physiological and pathological processes. To explore the involvement of miRNAs in the cytotoxicity of CdTe QDs, the SOLiD sequencing, a method that widely used to analyze miRNA expression profiling [25,26], was performed in this paper. The lengths of most SOLiD sequences were found to be distributed between 21 and 24 nucleotides (Fig. 3a), which was similar to the length distribution of registered miRNAs. The subsequent miRNA database-blasting confirmed that those sequences were mature miRNAs. We then analyzed the chromosome distribution of miRNAs detected in the SOLiD sequencing. Results showed that miRNAs detected in the SOLiD sequencing could be localized onto all chromosomes (Fig. 3b). And 45 types of miRNA were located onto chromosome 2, which had the greatest distribution of miRNAs.

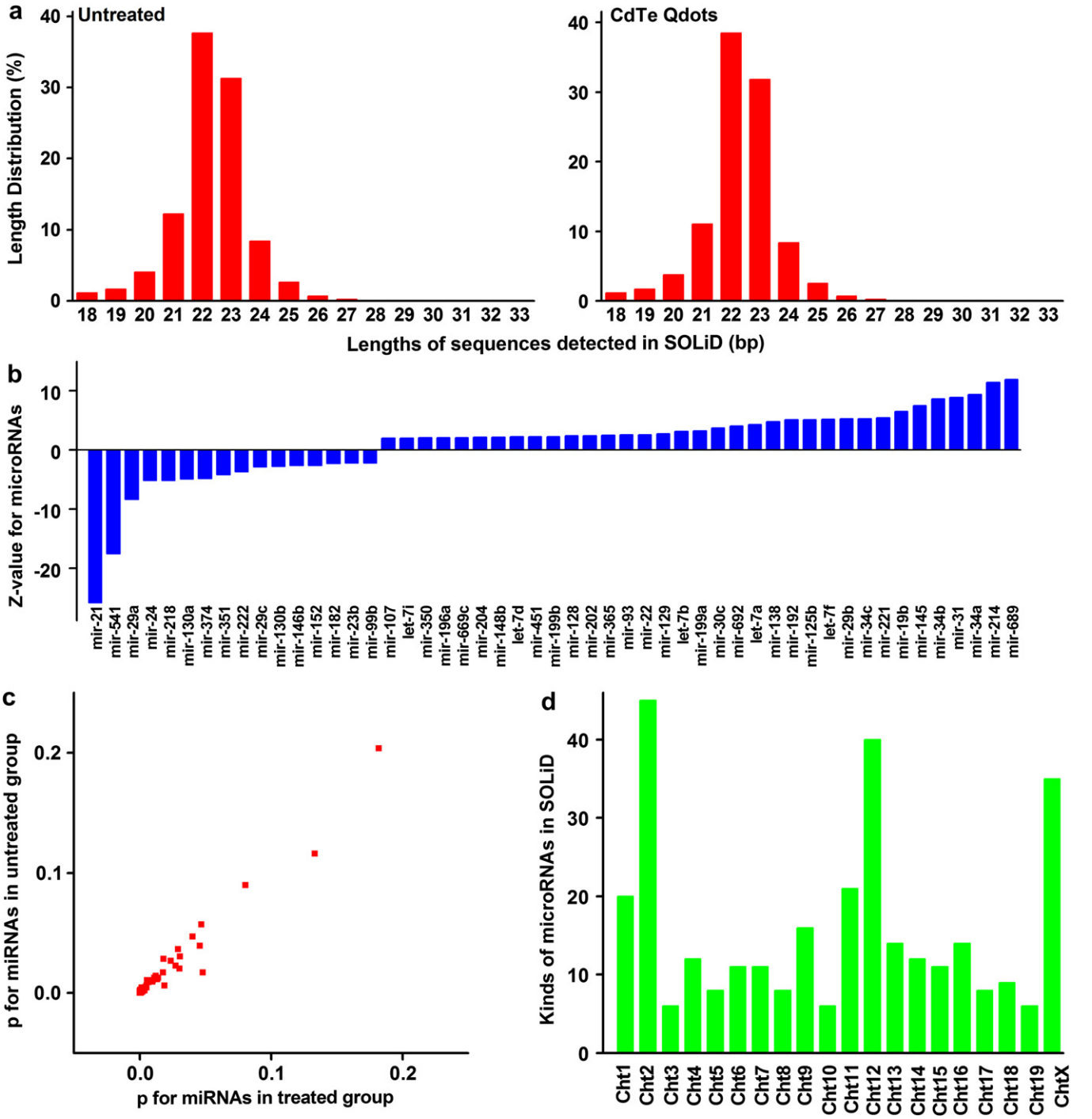

Fig. 3. Results of SOLiD sequencing. (a) Length distribution of sequences obtained from SOLiD sequencing. Sequences with 21–24 nucleotides length occupied the most part in all detected sequences. (b) miRNAs significantly regulated by CdTe QDs according to Z-test. (c) Scatter diagram of relationship between miRNA coverage of the control group and the CdTe QD treatment group. (d) Chromosome distribution of miRNAs revealed by SOLiD sequencing.

miRNAs can regulate the flow of genetic information by repressing the translation or stability of mRNAs without altering the primary DNA sequences [27–29]. By doing that, miRNAs are widely involved in the physiological or pathological processes. The fact that the apoptosis-like cell death was induced by CdTe QD exposure indicated that the expression levels of apoptosis-related proteins should be modulated. As important regulators in protein expression, miRNAs were involved in the translational controls of apoptosis-related proteins. The expression levels of the anti-apoptotic protein Bcl-2 protein can be repressed by miR-34 family miRNAs at the post-transcriptional level [30,31], which confirms the possibility of the involvements of miRNAs in the cytotoxicity of CdTe QDs.

3.3. Dysregulated miRNA expression after QD exposure

Compared with the drawbacks of the fold-change analysis, the Z-test was a better choice for analyzing SOLiD results, which granted the coverage of a specific miRNA per cell as a fraction or a proportion of the total number of miRNA molecules. The Z-test not only concerned changes in miRNA expression but also the coverage of miRNAs in a cell. Thus, miRNA expression patterns could be obtained by analyzing the results of SOLiD sequencing with the Z-test. The correlation between the miRNA coverage of the control group and the CdTe QD treatment group was lower than one (Fig. 3c), which meant that more miRNAs were up-regulated ($p_{test} - p_{control} > 0$, then the Z-value >0). In contrast to 86 miRNAs with down-regulated expression, the expression of 121 miRNAs were up-regulated by CdTe QD exposure. Among those miRNAs detected by the SOLiD sequencing, the expression of 16 and 35 miRNAs were down- and up-regulated by CdTe QD exposure, respectively. Therefore, the expression patterns of miRNAs were globally affected by CdTe QDs in NIH/3T3 cells.

The dysregulated expression of miRNAs can modulate the expression levels of their target mRNAs, which brings about variations in physiological or pathological processes. In other words, the expression patterns of miRNAs can reflect changes in cell statements. It was not inconceivable that miRNAs played important roles in the cytotoxicity of CdTe QDs. Although mature miRNAs are only ~22 nt long, the miRNA biogenesis is a complex affair and highly regulated. And it gives cells the capability of adapting to external stimuli through modulating the expression patterns of miRNAs. Therefore, the dysregulated expression of miRNAs is an adaptive process for cells.

3.4. miRNA expression with dose- and time-dependences

CdTe QDs can cause cell damage and death in a concentration-dependent manner in many cell lines [7,9,32]. Because of a disadvantage of SOLiD sequencing (high cost of sequencing), quantitative real-time PCR was applied to clarify the concentration-dependent cytotoxicity of CdTe QDs in NIH/3T3 cells. After incubation with changing concentrations of CdTe QDs, the expression of some significantly regulated miRNAs were detected with qRT-PCR. Results indicated that the expression of most miRNAs detected in this paper were affected by CdTe QDs in a dose-dependent manner (Fig. 4a). But the degree of dose-dependency on CdTe QDs for miRNA expression was lower than that for the cell survival ratios. That was because the changes in physiological and/or pathological processes were caused by the cooperation of all the miRNAs.

The correlation between expression levels of miRNAs detected with SOLiD sequencing and qRT-PCR was analyzed in this paper. Results of the two methods had significant relativity (Fig. 4b), which meant that qRT-PCR could represent SOLiD sequencing for analyzing the expression of a specific miRNA. In addition, the relationship between the expression levels of miRNAs at different exposure times (12 h and 24 h) was also analyzed. The results indicated that relationships of only four miRNAs (miR-29a, miR-93, miR-145 and miR-214) reached the significant statistical level (Fig. 4c). The differences among the expression levels of miRNAs were mainly caused by the distinctions in exposure times. For all miRNAs are not created equally and different mechanisms allow for the specific control of an individual miRNA, which is possible for miRNAs with changing expression levels under different external environments.

3.5. Pri-miRNA transcription as sources of dysregulated miRNA expression

The expression levels of miRNAs had been widely changed by CdTe QD exposure. And those changes in the expression levels of miRNAs are probably caused in the processes of miRNA biogenesis and maturation. Then, we explored the influences of CdTe QDs on the transcription processing of primary miRNAs. Results of qRT-PCR demonstrated that the transcription levels of pri-miRNAs were affected by the incubation with CdTe QDs (Fig. 5a and b). For miRNAs with up-regulated expression, the transcription of their relevant pri-miRNAs were promoted by CdTe QD incubation. In contrast, the transcription of pri-miRNAs for miRNAs with down-regulated expression were repressed.

In addition, the transcription of some pri-miRNAs chosen in this paper were in dose-dependent manners, which was similar to the expression of mature miRNAs. Although the discrepancies between the levels of pri-miRNAs and mature miRNAs were actually present, the transcription of pri-miRNAs made inevitable contributions to the expression levels of miRNAs. That was to say, the intricate means of transcriptional regulation of pri-miRNAs were affected by CdTe QD exposure. And the transcription of pri-miRNAs at different exposure times had no significant relationship, which meant that the transcription of pri-miRNAs didn't have time-dependent tendencies.

For the reason that the bulk of pri-miRNAs are transcribed by RNA polymerase II [33,34], the transcription of pri-miRNAs are accordingly subject to similar intricate means of the transcriptional regulation of genes [35]. Epigenetic control can play fundamental roles in the regulation of miRNA expression [36–39]. Researches on epigenetic effects of CdTe QDs have demonstrated that histone modification are affected by CdTe QD treatment [8,9], and then influence the transcription of genes and miRNAs. Thus, the dysregulated expression of miRNAs induced by CdTe QDs started from the first step involved in miRNA biogenesis, namely the transcription of pri-miRNAs.

3.6. Modulation of pri-miRNA transcription by p53

Except for the epigenetic controls described above, numerous RNA Polymerase II-associated transcription factors are widely involved in transcriptional regulation of miRNA genes. Among those transcription factors, *p53* is widely researched for its role as a tumor suppressor [40]. Then we explored the influences of CdTe QDs on the mRNA levels and the protein levels of *p53*. As we saw no significant changes in the mRNA levels of *p53* upon QD treatment (Fig. 6b), we asked whether there were changes in the protein levels of *p53* in CdTe QD treated cells. The western blotting results indicated that CdTe QDs induced a dramatic increase in the total protein levels of *p53* (Fig. 6a and c). After exposure to the same concentration of CdTe QDs at different times, the protein levels of *p53* at 24 h were higher than those at 12 h.

The post-translational modification of *p53* by phosphorylation has been proposed to be an important mechanism by which the stabilization and function of *p53* is regulated [41,42]. Among those forms of activated *p53* by phosphorylation, the protein levels of phosphorylated *p53* at Ser-15 were detected. Just as we had thought,

the protein levels of phosphorylated *p53* were raised by CdTe QD exposure (Fig.6a and c). Surprisingly, the coverage of phosphorylated *p53* in total *p53* was not significantly affected by the changing doses of CdTe QDs. A relationship analysis between the transcriptional levels of pri-miRNA and the protein levels of phosphorylated *p53* was performed. As no significant relation was found, the net accumulation of pri-miRNA was not only affected by the transcription regulation. And it meant that cleavage of pri-miRNAs by Drosa/DGCR8 was in sync with the transcription of pri-miRNAs.

CdTe QD treatment induced increase in overall *p53* protein levels without up-regulating the mRNA levels of *p53*. Upon cellular stress and DNA damage, *p53* was phosphorylated at the serine residues in the N-terminal, which was confirmed by the increased protein levels of phosphorylated *p53* at Ser-15. This activated form of *p53* is then freed from its nuclear binding complex, and binds DNA with higher affinity, thereby improving its efficiency as a transcription factor. Several reports have demonstrated that miR-34 family genes (miR-34a, miR-34b and miR-34c) are direct transactivation targets of *p53* and their induction promote apoptosis, cell cycle arrest, and senescence [43–45]. miR-34s seemed to complement the *p53* function through regulation of various targets involved in cell cycle and apoptosis. The results of qRT-PCR indicated that the transcriptional levels of miR-34s were up-regulated by CdTe QD exposure, which suggested the involvement of *p53* in the transcription of pri-miRNA.

3.7. Promotion of differential miRNA expression by pri-miRNA processing

Although the regulation of miRNA transcription is clearly important for setting a miRNA expression pattern, the transcriptional regulation is not the only layer of regulation. Many steps can be regulated in the mature processing of miRNAs, such as pri-miRNA processing by Drosha/DGCR8 and pre-miRNA processing by Dicer [46]. This paper analyzed the pri-miRNA processing by Drosha through detecting the expression levels of pre-miRNAs. Similar to the expression of pri-miRNAs, the expression of pre-miRNAs had dose-dependent tendencies (Fig.5c and d). But the correlations between the expression levels of pri-miRNAs and pre-miRNAs didn't reach the significant levels, which meant that the differential expression of pre-miRNAs were not strictly synchronous with those of pri-miRNAs.

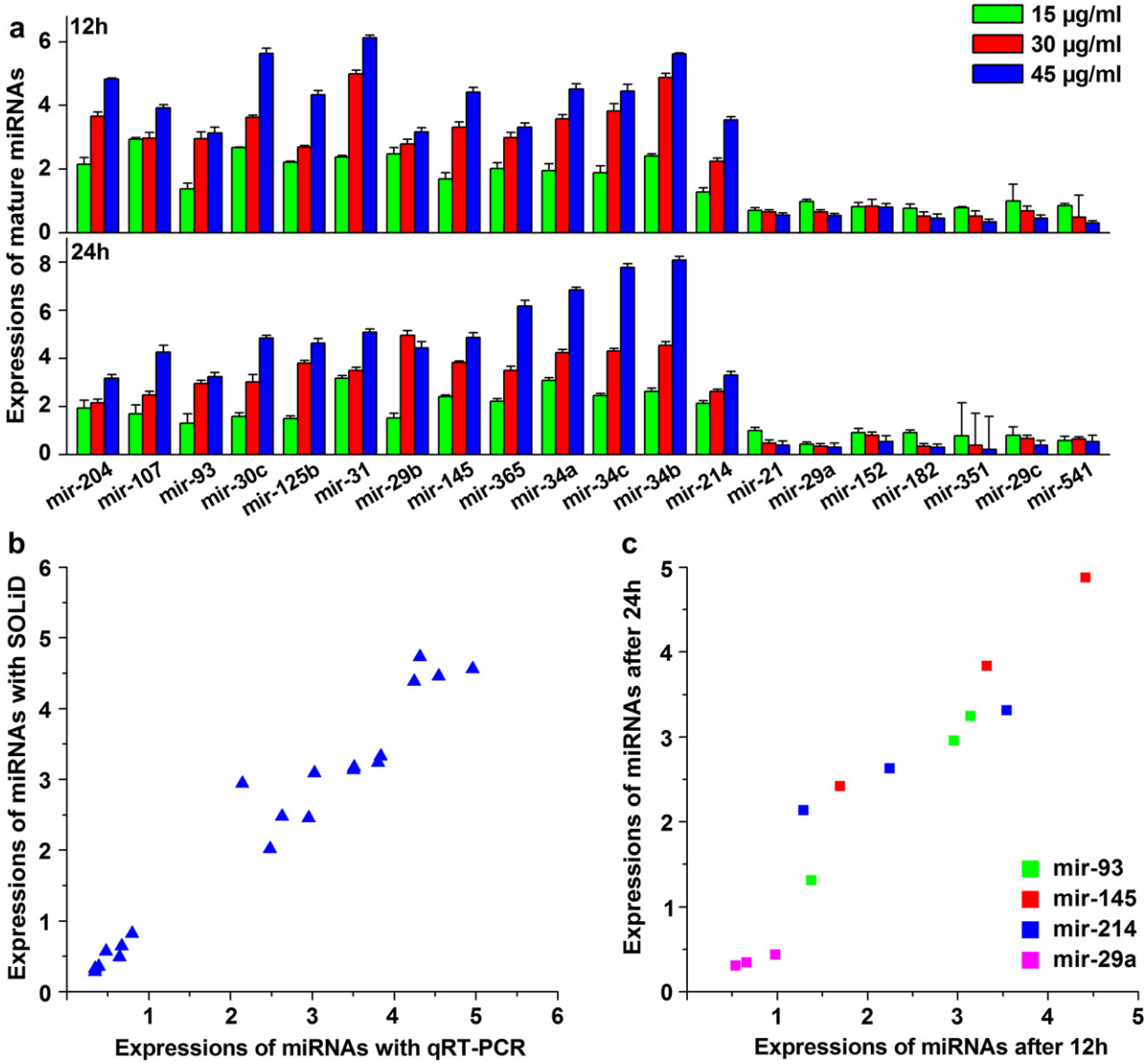

Fig. 4. Results of miRNAs expression analyzed by qRT-PCR. (a) Expression ratio of mature miRNAs detected with qRT-PCR after incubation with CdTe QDs at 12 h and 24 h, respectively; (b) Correlation between expression ratios of miRNAs detected with SOLiD and qRT-PCR; (c) Relationship between expression ratios of miRNAs at 12 h and 24 h.

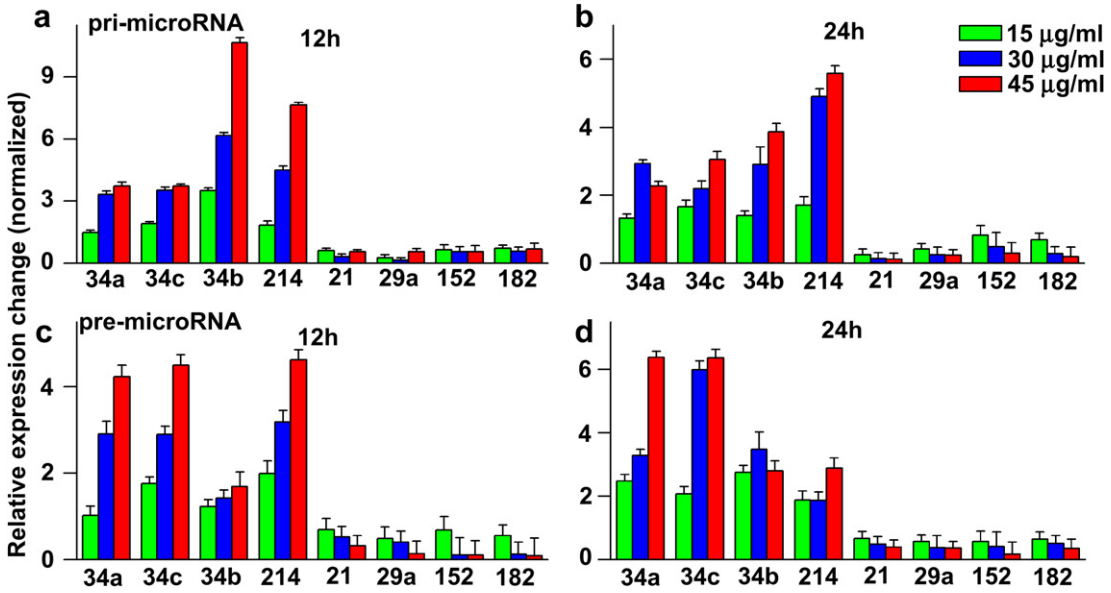

Fig. 5. Expression ratios of miRNA precursors after incubation with changing doses of CdTe QDs. (a) Expression ratios of pri-miRNAs under exposure to CdTe QDs for 12 h (a) and 24 h (b). Expression ratios of pre-miRNAs under exposure to CdTe QDs for 12 h (c) and 24 h (d).

The generations of pre-miRNAs, dsRNAs and miRNAs are enzyme catalyzed processes that are proportional to the concentrations of intermediates [47], which means that the mature processing of those intermediates can occur in the wake of their biogenesis. And the pri-miRNA processing can be accelerated by the activation of the transcription of pri-miRNAs. In addition, *p53* interact with the Drosha processing complexes through the association with DEAD-box RNA helicase *p*68 and facilitate the processing of pri-miRNAs to pre-miRNAs [15]. We found that the protein levels of *p53* were increased by CdTe QD exposure, which then could expedite the processing of pri-miRNAs to pre-miRNAs. And the non-significant correlation between the expression of pri-miRNAs and pre-miRNAs confirmed the dynamic changes.

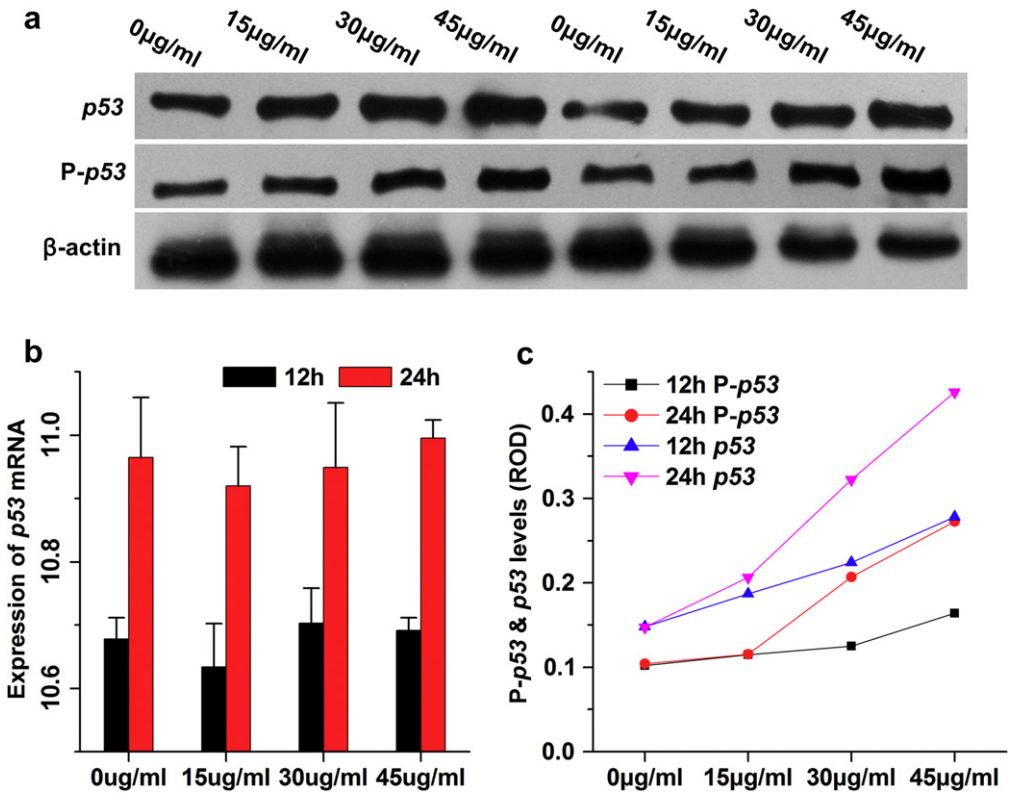

Fig. 6. *p53* was involved in the cytotoxicity of CdTe QDs. (a) Western blotting graphs of total *p53* and phosphorylated *p53* at Ser-15 under exposure to CdTe QDs for 12 h and 24 h; (b) Expression ratios of *p53* mRNA under exposure to CdTe QDs for 12 h and 24 h, respectively; (c) Relative protein levels of *p53* and Ser-15 phosphorylated *p53* under exposure to changing doses of CdTe QDs for 12 h and 24 h.

4. Conclusions

The expression patterns of miRNAs are globally altered by CdTe QDs in cells with apoptosis-like death, and that suggests a method with which to deeply assess the cytotoxicity of CdTe QDs. The dysregulated expression of miRNAs are mainly caused in the processing of miRNA biogenesis, which is a self-adjusting processing due to the external stimuli. The aberrant transcription of pri-miRNAs are conceivably performed in manners similar to the dysregulated expression of mature miRNAs. For the complexity of regulation in pri-miRNA processing, there are no significant linear relationships between the expression levels of pri-miRNAs and pre-miRNAs. In addition, the discrepancies between the levels of different processing intermediates and mature miRNAs are found in this paper, which means that there still are some indistinct regulatory mechanisms involved in the biogenesis of miRNAs.

Acknowledgments

This work was financially supported by the National Basic Research Program of China (973 Program: 2007CB936300), NSFC (No. 61071047, No. 30870626) and 2008DFA51180.

Appendix. Supplementary data

Supplementary data associated with this article can be viewed in the online version at doi:10.1016/j.biomaterials.2011.01.074.

References

[1] RiveraGil P, Oberdorster G, Elder A, Puntes V, Parak WJ. Correlating physicochemical with toxicological properties of nanoparticles: the present and the future. ACS Nano 2010;4:5527–31.
[2] Weiss PS. The big picture. ACS Nano 2009;3:1603–4.
[3] Cho SJ, Maysinger D, Jain M, Roder B, Hackbarth S, Winnik FM. Long-term exposure to CdTe quantum dots causes functional impairments in live cells. Langmuir 2007;23:1974–80.
[4] Zhang Y, Mi L, Xiong R, Wang PN, Chen JY, Yang W, et al. Subcellular localization of thiol-capped CdTe quantum dots in living cells. Nanoscale Res Lett 2009;4:606–12.
[5] Su Y, Hu M, Fan C, He Y, Li Q, Li W, et al. The cytotoxicity of CdTe quantum dots and the relative contributions from released cadmium ions and nanoparticle properties. Biomaterials 2010;31:4829–34.
[6] Prasad BR, Nikolskaya N, Connolly D, Smith TJ, Byrne SJ, Gerard VA, et al. Long-term exposure of CdTe quantum dots on PC12 cellular activity and the determination of optimum non-toxic concentrations for biological use. J Nanobiotechnology 2010;8:7.
[7] Lovric J, Cho SJ, Winnik FM, Maysinger D. Unmodified cadmium telluride quantum dots induce reactive oxygen species formation leading to multiple organelle damage and cell death. Chem Biol 2005;12:1227–34.
[8] Conroy J, Byrne SJ, Gun'ko YK, Rakovich YP, Donegan JF, Davies A, et al. CdTe nanoparticles display tropism to core histones and histone-rich cell organelles. Small 2008;4:2006–15.
[9] Choi AO, Brown SE, Szyf M, Maysinger D. Quantum dot-induced epigenetic and genotoxic changes in human breast cancer cells. J Mol Med 2008;86:291–302.
[10] Singh N, Manshian B, Jenkins GJ, Griffiths SM, Williams PM, Maffeis TG, et al. NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. Biomaterials 2009;30:3891–914.
[11] Myllynen P. Nanotoxicology: damaging DNA from a distance. Nat Nanotechnol 2009;4:795–6.
[12] Petersen EJ, Nelson BC. Mechanisms and measurements of nanomaterial induced oxidative damage to DNA. Anal Bioanal Chem 2010;398:613–50.
[13] Maysinger D. Nanoparticles and cells: good companions and doomed partnerships. Org Biomol Chem 2007;5:2335–42.
[14] Iorio MV, Piovan C, Croce CM. Interplay between microRNAs and the epigenetic machinery: an intricate network. Biochim Biophys Acta 2010;1799:694–701.
[15] Suzuki HI, Yamagata K, Sugimoto K, Iwamoto T, Kato S, Miyazono K. Modulation of microRNA processing by *p53*. Nature 2009;460:529–33.
[16] Xing B, Li WW, Sun K. A novel synthesis of high quality CdTe quantum dots with good thermal stability. Mater Lett 2008;62:3178–80.
[17] Wang Y, Zheng JW, Zhang ZJ, Yuan CW, Fu DG. CdTe nanocrystals as luminescent probes for detecting ATP, folic acid and L-cysteine in aqueous solution. Colloid Surf A 2009;342:102–6.
[18] Kal AJ, van Zonneveld AJ, Benes V, van den Berg M, Koerkamp MG, Albermann K, et al. Dynamics of gene expression revealed by comparison of serial analysis of gene expression transcript profiles from yeast grown on two different carbon sources. Mol Biol Cell 1999;10:1859–72.
[19] Ruijter JM, Van Kampen AH, Baas F. Statistical evaluation of SAGE libraries: consequences for experimental design. Physiol Genomics 2002;11:37–44.
[20] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. Methods 2001;25:402–8.
[21] Ngamwongsatit P, Banada PP, Panbangred W, Bhunia AK. WST-1-based cell cytotoxicity assay as a substitute for MTT-based assay for rapid detection of toxigenic Bacillus species using CHO cell line. J Microbiol Methods 2008;73:211–5.
[22] Samia AC, Chen X, Burda C. Semiconductor quantum dots for photodynamic therapy. J Am Chem Soc 2003;125:15736–7.
[23] Su YY, He Y, Lu HT, Sai LM, Li QN, Li WX, et al. The cytotoxicity of cadmium based, aqueous phase – synthesized, quantum dots and its modulation by surface coating. Biomaterials 2009;30:19–25.
[24] Jan E, Byrne SJ, Cuddihy M, Davies AM, Volkov Y, Gun'ko YK, et al. High-content screening as a universal tool for fingerprinting of cytotoxicity of nanoparticles. ACS Nano 2008;2:928–38.
[25] Qi Y, Tu J, Cui L, Guo X, Shi Z, Li S, et al. High-throughput sequencing of microRNAs in adenovirus type 3 infected human laryngeal epithelial cells. J Biomed Biotechnol 2010;2010:915980.
[26] Schulte JH, Marschall T, Martin M, Rosenstiel P, Mestdagh P, Schlierf S, et al. Deep sequencing reveals differential expression of microRNAs in favorable versus unfavorable neuroblastoma. Nucleic Acids Res 2010;38:5919–28.
[27] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.
[28] Ambros V. The functions of animal microRNAs. Nature 2004;431:350–5.
[29] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834–8.
[30] Hermeking H. *p53* enters the microRNA world. Cancer Cell 2007;12:414–8.
[31] Ji Q, Hao X, Meng Y, Zhang M, Desano J, Fan D, et al. Restoration of tumor suppressor miR-34 inhibits human *p53*-mutant gastric cancer tumorspheres. BMC Cancer 2008;8:266.
[32] Zhang Y, Chen W, Zhang J, Liu J, Chen G, Pope C. *In vitro* and *in vivo* toxicity of CdTe nanoparticles. J Nanosci Nanotechnol 2007;7:497–503.
[33] Cai X, Hagedorn CH, Cullen BR. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA 2004;10:1957–66.
[34] Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, et al. MicroRNA genes are transcribed by RNA polymerase II. EMBO J 2004;23:4051–60.
[35] Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol 2009;10:126–39.
[36] Chuang JC, Jones PA. Epigenetics and microRNAs. Pediatr Res 2007;61:24R–9R.
[37] Agirre X, Vilas-Zornoza A, Jimenez-Velasco A, Martin-Subero JI, Cordeu L, Garate L, et al. Epigenetic silencing of the tumor suppressor microRNA hsa-miR-124a regulates CDK6 expression and confers a poor prognosis in acute lymphoblastic leukemia. Cancer Res 2009;69:4443–53.
[38] Bueno MJ, Perez de Castro I, Gomez de Cedron M, Santos J, Calin GA, Cigudosa JC, et al. Genetic and epigenetic silencing of microRNA-203 enhances ABL1 and BCR-ABL1 oncogene expression. Cancer Cell 2008;13:496–506.
[39] Toyota M, Suzuki H, Sasaki Y, Maruyama R, Imai K, Shinomura Y, et al. Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in colorectal cancer. Cancer Res 2008;68:4123–32.
[40] Levine AJ. The *p53* tumor-suppressor gene. N Engl J Med 1992;326:1350–2.
[41] Meek DW. Post-translational modification of *p53*. Semin Cancer Biol 1994;5:203–10.
[42] Ashcroft M, Kubbutat MH, Vousden KH. Regulation of *p53* function and stability by phosphorylation. Mol Cell Biol 1999;19:1751–8.
[43] Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, et al. Transactivation of miR-34a by *p53* broadly influences gene expression and promotes apoptosis. Mol Cell 2007;26:745–52.
[44] Corney DC, Flesken-Nikitin A, Godwin AK, Wang W, Nikitin AY. microRNA-34b and microRNA-34c are targets of *p53* and cooperate in control of cell proliferation and adhesion-independent growth. Cancer Res 2007;67:8433–8.
[45] He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, et al. A microRNA component of the *p53* tumour suppressor network. Nature 2007;447:1130–4.
[46] Zeng Y. Principles of microRNA production and maturation. Oncogene 2006;25:6156–62.
[47] Wang X, Li Y, Xu X, Wang YH. Toward a system-level understanding of microRNA pathway via mathematical modeling. Biosystems 2010;100:31–8.