The radioresistance analysis of the *Deinococcus radiodurans* gene DR1709 in human bone marrow cell line KG1a

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Accepted 16 September, 2011

Tumors continued to grow in many cancer patients after they received radiation therapy, which required higher radiation dose. But when high dose of radiation damaged tumor cells, some normal cells were also killed, which was the largest obstacle that doctors cannot use high dose radiation. If the radiation resistant genes were transformed into the normal tissues, the normal tissues would be protected from being damaged when patients were irradiated. The tolerating doses of all radioresistant (radiation-resistant) genes for this aim were lower than clinical requirement. There were many strong radiation resistant genes in *Deinococcus radiodurans* genome. If these genes can play their radiation resistant roles in human cells, the predicaments that radiation therapy faced now would be conquered. DR1709 was a radiation resistant gene from *D. radiodurans*. We found that *Escherichia coli* transformed with DR1709 had much higher radioresistance than the control. In this paper, human bone marrow cell line, KG1a, was transformed with DR1709. Cells contained DR1709 and the control cells were treated with gamma radiation. Results showed that cells containing DR1709 had much higher survival fraction than those cells which were only transformed with the net vector. After being transformed with DR1709, the expression of GLRX2 was induced in KG1a, while the expression of the putative holocytochrome c-type synthetase was suppressed. Genes from *D. radiodurans* can be used as a shield for normal cells when they are irradiated. But there were many questions to be resolved before this result was used in clinical treatment.

Key words: Radioresistance, DR1709, KG1a, mitochondria.

INTRODUCTION

*Deinococcus radiodurans* was one of the most radiation-resistant biology (Makarova et al., 2001). It can survive after being irradiated with 60 Gy. Even treated with 15 kGy acute irradiation, it still has some survival rate (Daly and Minton, 1996). Some most radiation resistant genes might be contained in *D. radiodurans*’ genome (*D. radiodurans*’ genome might contain some very strong radiation-resistant genes). Hua et al. (2003) isolated a switch gene, ppri, which controlled the downstream repairs from a natural mutant and when *D. radiodurans* was irradiated, ppri markedly induce the expression of recA and pprA, promoting the high-efficiency repairs of the damaged DNA. These genes can regulate DNA repair through upstream controlling, and contributing to this bacterium’s radiation resistance (Wang et al., 2008; Chen et al., 2008).

After DR1709 was disrupted, the mutant and the original *D. radiodurans* cells were treated with UV radiation. The survival of the mutant was much lower than the wild type (Chang et al., 2009). When *Escherichia coli* were transformed with DR1709, the transformed cells had higher radiation resistance than the original strain (Shu and Tian, 2010). These results showed that DR1709 not only can play strong radiation radiance in *D. radiodurans*, but also can play similar roles in the genomic background which was much more different from its native strain. If this gene can still play such role in eukaryote cells, it may be utilized in radiation protection.

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The recombinant gene to be transformed. The italic capital letters showed the mitochondria leading sequence. The small letters referred to the sequence of DR1709. The starting code and the terminal code were shown by pane. PolyA was shown with boldface-capital letters.

Figure 1. The recombinant gene to be transformed.

The leading sequence and polyA of human MnSOD
dose used (10, 40 and 80 Gy) was determined by the distance
tube with 0.3 mg/ml EDTA, 0.7 μg/ml leupeptin). The
hepatoma leading sequence and polyA of human MnSOD.
the expression of GLRX2 (Glutaredoxin-2, mitochondrial) was induced,
and radiation therapy. To verify whether this gene can
play such roles, in this paper. DR1709 was ligated with
mitochondria leading sequence and polyA (Figure 1). The
recombinant gene was transformed into the human bone
marrow cell line KG1a. Results showed that KG1a
containing DR1709 had much higher survival than the
original KG1a when treated with gamma radiation. After
being transformed with DR1709, the expression of
GLRX2 (Glutaredoxin-2, mitochondrial) was induced,
while the expression of the putative holocytochrome c-
type synthetase was suppressed.

MATERIALS AND METHODS

Plasmids and strains

pBABEhygro-Cdc42L61 was bought from Biolab (Beijing, China).
KG1a and PA317 were bought from Shanghai Biosis Biotechnology
limited company (Shanghai, China). Cell culture and transferring
were mainly according to the method described previously (Zhang
et al., 2003b) were synthesized by Biolab company (Beijing, China).
DR1709 was isolated from the genome of D. radiodurans according
to our previous paper (Shu and Tian, 2010). The three sequences
were ligated using T4 DNA ligase (TaKaRa, Dalian, China). The
ligated product was inserted into pMD19-T vector (TaKaRa, Dalian,
China). The recombining plasmid was isolated using E. coli
JM109 (TaKaRa, Dalian, China). The recombinant plasmid was sequenced by Shanghai Biosis Biotechnology limited company
(Shanghai, China). The plasmid was digested and subcloned into
pBABEhygro-Cdc42L61. The retrovirus vector was constructed and
named as pBABDR1709.

Determination of G418 concentration

Different content of G418 (100, 200, 300, 400, 500, 600, 700, 800,
900 and 1000 μg/ml respectively) was loaded into the cell medium.
Cells were cultured as described earlier. The medium was replaced
in every 2 days. The content that all of the cells died
on the 14th day were selected as the lowest concentration.

Amphotropic retrovirus cell PA317 was transformed with
pBABDR1709 thus, generating virus cell. Cells transformed with the
net vector, pBABEhygro-Cdc42L61 were used as control. Using
KG1a as the received cell, the supernatant tittering was measured.

Extraction of soluble proteins

The transformed KG1a cells and non-transformed KG1a cells were collected by centrifugation respectively. The pellets were
resuspended in phosphate buffer (1 mol/L, pH 7.0). The suspension
was mixed with four times the volume of Lysis buffer (9 mol/L urea,
4% mass concentration CHAPS, 1% mass concentration DTT, 0.5%
leupeptin). The cells in the mixture were sonicated in 0°C bath with half-time intermittent (Branson, Sonifier 450) for 1 h. The homogenates were
Two-dimensional gels electrophoresis

The procedure of the two-dimensional gels electrophoresis was performed according to the standard method (Pelroy and Whiteley, 1971). For each channel, 350 μg of purified sample was diluted with rehydration buffer (8 mol/L urea, 2% CHAPS, 0.5% IPG buffer, 0.002% bromphenol blue) and loaded onto the IPG strip (24 cm, covering the pH ranges 47; Amersham Biosciences, USA) and the isoelectric focusing was performed (50 V, 12 h; 200 V, 2 h; 500 V, 1 h; 1500 V, 30 min; 8000 V, 6 h). And then, the strips were treated with equilibration buffer I (6 mol/L urea, 30% glycerol, 2% SDS, 50 mmol/L Tris-HCl, 1% DTT, 0.002% bromphenol blue) and equilibration buffer II (6 mol/L urea, 30% glycerol, 2% SDS, 50 mmol/L Tris-HCl, 4% iodoacetamide, 0.002% bromphenol blue) respectively. The second dimensional electrophoresis was performed in 12.5% SDS-PAGE gels (5 W/gel, 30 min; 60 W/gel, 10 h). The gels were stained with a modified Neuhoff’s colloidal Coomassie Blue G-250 stain (Candiano et al., 2004; Neuhoff et al., 1988).

Protein spots identification

Protein samples were in-gel digested and identified according to the standard method (Sun et al., 2007). The work of MALDI-TOF-TOF MS and peptide mass fingerprinting was performed by Tianjin Biochip Corporation (Tianjin, China).

RESULTS

Construction of the retrovirus vector

After the leading sequence, DR1709 and polyA were ligated, the product was inserted into pMD19-T. The plasmid was transformed into E. coli. After being extracted from E. coli, the plasmid was digested and sequenced (Figure 2). Results showed that the three fragments had been ligated successfully.

DNA was extracted from the transformed cells. PCR was performed with primers corresponding to the recombinant gene (Figure 3). In 1% agarose gel, transformed cell had one band whose molecular weight was about 1.6 kb, while no band could be found in non-transformed cell, showing that KG1a had been transformed successfully.

Cells’ sensitivity to irradiation

Non-transformed KG1a cell, KG1a cell transformed with vector pBABEhygro-Cdc42L61 and the KG1a cell transformed with pBABDR1709 was irradiated with gamma radiation respectively. When the radiation dose was 10 Gy, the survival of non-transformed KG1a cell and KG1a cell transformed with vector pBABEhygro-Cdc42L61 centrifuged at 12000 × g for 20 min at 4°C. The supernatants were collected into a 1.5-microtube and used as a soluble protein fraction. Protein samples were digested and identified according to the standard method (Sun et al., 2007). The work of MALDI-TOF-TOF MS and peptide mass fingerprinting was performed by Tianjin Biochip Corporation (Tianjin, China).
were similar. The corresponding survival fraction was 10% and 10.5%. But KG1a cell transformed with pBABDR1709 had much higher survival than the previous two lines (Figure 4). Its survival fraction was about 43% (Figure 4). When irradiation dose increased, the survivals of these three cell lines were less than their corresponding data under 10 Gy. But the survival of KG1a cell line transformed with pBABDR1709 was still much higher than the other two lines (Figure 5). These indicated that after being transformed into (with) DR1709, KG1a cell had higher radiation resistance than the original cell line.

3 proteomic analysis of the KG1a cell lines

To investigate what had happened in KG1a after it was transformed, two-dimensional electrophoresis was performed. Results demonstrated that at least three protein dots' intensities changed remarkably. Protein 1 and protein 2 were characterized using MALDI-TOF-TOF MS, showing that they were GLRX2 (Glutaredoxin-2, mitochondrial) and putative holocytochrome c-type synthetase. After being transformed with DR1709, the expression of GLRX2 was induced in KG1a, while the expression of the putative holocytochrome c-type synthetase was suppressed.

DISCUSSION

Cancer patients had high reoccurrence rates after they received radiation therapy. Higher dose of radiation was required to resolve this problem. The major obstacle preventing the delivery of higher irradiation doses is normal tissue toxicity (Epperly et al., 2002a; Greenberger, 2003). If some certain gene was transformed into the normal tissue to improve their survival fraction, this problem can be resolved (Greenberger, 1999). Plasmids contained the gene MnSOD were injected into mouse esophagus. The mouse lungs were irradiated using 35 Gy for 24 h. The mouse being injected had higher survival than the contrasting (Epperly, 2002b). Though these results indicated that gene therapy had some certain potential use in radiation therapy, the irradiation doses used in those experiments were lower than those being used in clinical therapy (45 to 50 Gy).

DR1709 was a radiation-resistant gene isolated from *D. radiodurans*. After being transformed with DR1709, *E. coli* had higher resistance to γ and UV radiation (Shu and Tian, 2010). Treated with 150 Gy γ radiations, *E. coli* cells transformed with DR1709 had 70% survival fraction, while only 17% of the corresponding cells without DR1709 could be found on LB plate (Shu and Tian, 2010). When DR1709 was transformed into KG1a, KG1a cell had much higher survival than the original cell line. At
the dose of 50 Gy, the transformed cell’s survival fraction was still about 28%, which was about eight times as much as the original cell line. These results suggested that the effect of radiation therapy could be improved by transforming DR1709 into normal cells.

When animal cells were irradiated, superoxide radicals were produced, which attacked DNA strands, resulting in DNA strands broke (Marples, 2004; Marples et al., 2004). TP53 protein was activated by DNA breaks (Epperly et al., 2002c). BAX and SAP kinase migrated into mitochondria (Epperly et al., 2002c). Cytochrome C was released from mitochondria activating caspase 3 and ADP polymerase, and finally, the cells died (Epperly et al., 2002c; 2003a; 2003b; Zhang et al., 2008). Superoxide radicals were the main reason leading to cells death when cells were irradiated. Mitochondrial localization has been demonstrated to be critical to protect the cell from being damaged by superoxide radicals (Greenberger et al., 2003). Therefore, mitochondrial leading sequence was ligated with DR1709 in this paper.

To investigate the concrete mechanism that KG1a cell contained, DR1709 had higher radiation resistance than the original cell line, two-dimensional electrophoresis was performed. After being transformed with DR1709, the expression of GLRX2 was induced in KG1a cell, while the expression of the putative holocytochrome c-type synthetase was suppressed.

GLRX2 facilitates the maintenance of mitochondrial redox homeostasis upon induction of apoptosis by oxidative stress (Lundberg et al., 2001). It involved in response to hydrogen peroxide and regulation of apoptosis (Johansson et al., 2004). In KG1a containing DR1709, the expression of GLRX2 was much higher than the original cell line. GLRX2 catalyzes both glutathionylation and deglutathionylation of mitochondrial complex I, which in turn regulates the superoxide production by the complex (Johansson et al., 2004). In the transformed cells, GLRX2’s induction decreased the susceptibility to apoptosis, resulting that the survival fraction was improved.

C-type cytochromes are proteins that are essential for life. Superoxide radicals arise from the autoxidation of respiratory dehydrogenases, where adventitious transfer of electrons from reduced flavins (FADH2) associated with c-type cytochromes (Ghosal et al., 2005). It was thought that the total intracellular titer of cytochromes was a marker for the proclivity of cells to generate oxidative stress (Messner and Imlay, 2002). In the transformed KG1a cells, the expression of the putative c-type cytochrome was repressed. Less caspase 3 and ADP polymerase were activated (Epperly et al., 2002c; 2003a; 2003b; Zhang et al., 2008) and then, more cells survived.

REFERENCES


