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Mechanism of exogenous nucleic acids and their precursors improving the repair of intestinal epithelium after γ-irradiation in mice

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Abstract

AIM To clone expressed genes associated with repair of irradi ation-damaged mice intestinal gland cells treated by small intestinal RNA, and to explore the molecular mechanism of exogenous nucleic acids improving repair of intestinal crypt.

METHODS The animal mode of test group and control group was established, forty-five mice being irradiated by γ ray were treated with small intestinal RNA as test group, forty mice being irradiated by γ ray were treated with physiological saline as control group, five mice without irradiation were used as normal control, their jejunal specimens were collected respectively at 6h, 12h, 24h, 4d and 8d after irradiation. Then by using LD-PCR based on subtractive hybridization, these gene fragments differentially expressed between test group and control group were obtained, and then were cloned into T vectors as well as being sequenced. Obtained sequen ces were screened against. GeneBank, if being new sequences, they were submitted to GeneBank.

RESULTS Ninety clones were associated with repair of irradiation-damaged intestinal gland cells treated by intestinal RNA. These clones from test group of 6 h, 12 h, 24 h, 4 d and 8 d were respectively 18, 22, 25, 13, 12. By screening against GeneBank, 18 of which were new sequences, the others were dramatically similar to the known sequences, mainly similar to hsp, Nmi, Dutt1, alkaline phosphatase , homeobox, anti-CEA ScFv antibody, arginine/ serine kinase and BMP-4,repA. Eighteen gene fragments were new sequences,their accept numbers in GeneBank were respectively AF240164-AF240181.

CONCLUSION Ninety clones were obtained to be associated with repair of irradiation-damaged mice intestinal gland cells treated by small intestinal RNA, which may be related to abnormal expression of genes and matched proteins of hsp, Nmi, Dutt1, Na, K-ATPase, alkalineph- osphatase, glkA, single stranded replicative centromeric gene as well as 18 new sequences.

INTRODUCTION

After exposure to large dose ionizing radiations, the larger intestinal gland cell lesion is the main cause of death in humans and animals. How to enhance intestinal gland cell survival rates has become a problem to be solved. In order to increase the mouse crypt survival after irradiation, we have done a series of experiments, and finally confirmed that when a portion of the crypts was devast ated by irradiation, a compensatory recovery of the intestinal epithelium by remaining crypts occurred involving three consecutive periods such as the rapid cell proliferation of the viable crypts, the fission of the proliferative crypts and the increase of crypt numbers[1]. The nucleic acid fragment contai ning several hundred base pairs (bp), or even any one of the nucleic acid precursors (mononucleotides, nucleosides, and bases) can enhance the crypt survival rate by 25% or so, further confirming that the effectiveness of exogenous nucleic acids depends not upon the action exerted by their highly polymerized state, but upon their various enzymatic degradation products[2]. Our experimental results suggest that the nucleic acids (DNA, RNA) and their precursors may be used as one of the effective measures for the treatment of intestinal radiation

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syndrome that may occur in the war time as well as in the peaceful use of atomic energy[3-5]. However, the molecular mechanism how nuclear acids and precursors improve the repairing of irradiation-damaged intestinal gland cells is unclear. In order to clarify this molecular mechanism, we used long distance-PCR based on subtractive hybridization, isolated and cloned these genes associated with repairs of irradiation-damaged mice intestinal gland cells treated by intestinal RNA. Our studies lay foundation for further clarifying molecular mechanism of repair of radiation-damaged crypt.

MATERIALS AND METHODS

Reagents

PolyATtract® system 1000 kit from Promega was used for extraction of mRNA, SMART PCR cDNA synthesis kit (Clontech) for transcription of mRNA, Wizard® plus Minipreps DNA purification for purification of PCR production, Advantage2PCR kit (Clontech) for LD-PCR, and PE-5700 quantiative PCR cycler used for thermal cycle. PGEM-T easy vector system was purchased from Promega, γ-^{32}-pdATP from Beijing Fu Rei Compancy, and the other reagents were from Beijing Yuan Ping compancy.

Establishment of the model and sample collection

Ninety BALB/c male mice with body weight of 18g-22g and 10-12 weeks old were randomly assigned into two groups, *i.e,* test group and control group. They were injected with 5g·L^{-1} barbiturate sodium 40 mg·kg^{1}, put in organism radiation box, and then were irradiated on mice abdominal region by using ^{60}Co γ ray at the reagent rate of 149.47-151.13cGy·min^{-1}, and finally reached total reagent of 1150cGy. Small intestinal RNA was diluted into 100mg·mL^{-1}. Two hours after mice being irradiated, each mouse in test group was injected 0.4mL RNA liquid, and each mouse in control group was injected 0.4mL physiological saline by using local intestinal cavity expanding injection method[6]. After that, the mice were raised according to conventional method. These mice were killed respectively at 6 h, 12 h, 24 h, 4 d and 8 d after irradiation, and jejunal tissues were quickly taken out, washed by physiological saline, and then were kept in liquid nitrogen. In order to avoid single difference, these samples were mixed together under identical condition.

Sample processing and LD-PCR based on subtractive hybridization

Extraction of mRNA was done according to manual of PolyATtract system 1000, and quantificated. mRNA transcription was done according to manual from SMART PCR cDNA synthesis kit. Subtractive hybridization between test group and control group was done as follows: take out 0.1μg mRNA from the control group, add Biotinylated oligo(dT) probe (50μmol ·L^{-1}), 70℃ 5min, then add into the first strand cDNA, and hybridize for 24 h at 42℃, finally add Streptavidin magnesphere particles and mixed, magnetic steel was used to attract production of two stranded hybrids and get rid of them, and then repeat the processing twice. Take out the upper liquid, and add double volume absolute alcohol to the upper liquid to precipitate cDNAs, finally dissolve them in 10μL Nuclease-free water. Take 10μL of the first strand cDNA as template, sequentially add PCR-grade water 74μL, 10× advantage 2 PCR buffer 10μL, 50×dNTP mix-10μL, 50×advantage 2 polymerase mix 1μL, PCRprimer Mix 2μL, 50mmol·L^{-1} $MgCl_2$ 3μL, mixed well, centrifuged for several seconds, using two steps such as 95℃ 1min, 95℃ 15s, 68℃ 6min, 30cycles, in the end elongated at 68℃ for 6min, take out 10 μL production to run 12 g·L^{-1} agarose gel electrophoresis.

Cloning and identification of PCR products

PCR products were purified using Wizard plus Minipreps DNA purification system, and dissolved in 10μL water. According to the manual, PCR products were cloned into PGEM-T easy vector, transferred into JM109, positive clone picked up, cultured overnight, plasmid extracted out, and identified by cutting with BstZ1. Products cut by BstZ1 were labelled γ-^{32}pdATP at terminal, and used as probe, and hybridized with mRNA of test group and control group[7].

Sequencing and screening against GeneBank

These obtained clones were positively sequenced by using Model 377 sequencing instrument. Obtained sequences were screened against GeneBank, if being new sequences, these sequences were submitted to GeneBank[8].

RESULTS

LD-PCR based on subtractive hybridization

In test group of 6 h, 12 h, 24 h, 4 d and 8 d, products after subtractive hybridization were successfully amplified by LD-PCR, obtained bands centered on 1-1.5kb or so (Figure 1).

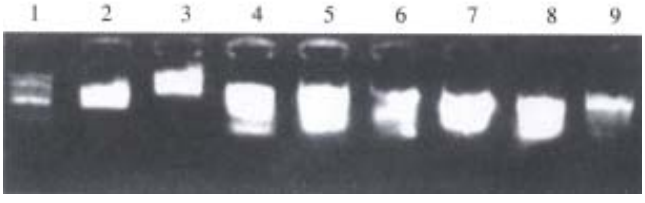

Figure 1 Electrophoresis result of production of LD-PCR.
1: Marker; 2, 6: Positive control; 3, 4, 5, 7, 8: Results of 6h,

12h, 24h, 4d and 8d; 9: Negative control

Cloning and identification of PCR products (Figure 2)

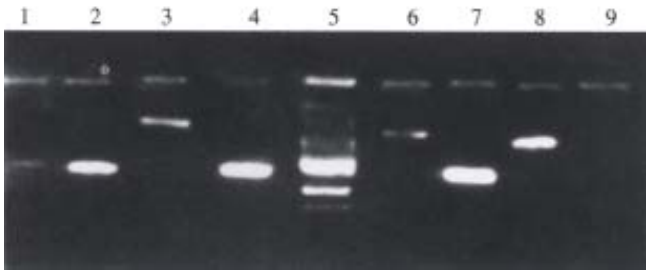

Figure 2 Results of cloning and identification of production of PCR. 1-4, 6-9: Results of part products cut by Bstz1; 5: Marker Ninety of positive clones were obtained from test group , positive clones of 6 h, 12 h, 24 h, 4 d and 8 d in test group were respectively eighteen, twenty-two, twenty-five, thirteen, twelve.

Identification of hybridization (Figure 3)

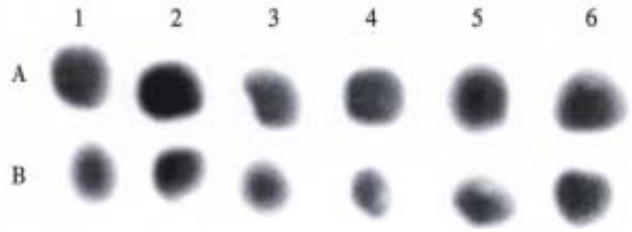

Figure 3 Part results of hybridization with RNA.
A: Test group; B: Control group
Dot blots confirmed that these genes were overexpressed in test group, and lower expressed in control group, that is, these genes were associated with repair of intestinal gland cells treated by RNA.

Sequencing and searching for GeneBank

These clones were sequenced according to the results of sequencing and searching against GeneBank, eighteen were new sequences, eighty-two were dramatically similar to the known sequences.

In test group of 6h, similar sequences mainly were as follows: mRNA for heat shock protein, Nmi mRNA, Dutt1 protein, mRNA for Na, K-ATPase gamma subunit, mRNA for surface glycoprotein, Zinc finger type transcript factor, porcine growth hormone-releasing hormone gene, monocyte/macrophage Igrelated gene, telomerase-associated protein, HOX1b protein, arginine/ serine kinase.

In test group of 12h, similar sequences were: Alkaline phosphatase mRNA, alkaline phosphatase 2, glkA gene, single stranded replicative centromeric gene, DMBT1, tRNA-Met gene, homeobox protein, thyroxine-binding globulin gene, alpha-a-plasmin inhibitor gene.

In test group of 24h, similar sequences were: anti-CEA ScFv antibody, anti-DNA heavy chain, mRNA for Ig kappa chain, anti BONT/A Hc ScFv antibody, mRNA for collagenase, AE0199 immunoglobulin heavy chain, Mouse Ig gamma-chain, Ig rearranged gamma-chain mRNA, anti -c-myc antibody, anti-CD30 moab ki_4 ScFv,anti-BSA antibody, D1 heavy chain, epidermal growth factor, anti-NP antibody IgH, mRNA for arginine/ serine kinase.

In test group of 4d, simliar sequences were: Dual specificity phosphatase, family mRNA telomerase-associated protein, anti-human erbB-2, tazarotene-induced gene2, betaine-GABA transporter gene, copy complex subunit 7a, mRNA for stress-activated protein, FK506 binding protein, calium/ calmodulin dependent gene, PEST phosphatase interactin gene, haptoglobin mRNA, acyl-ACP desaturase, mRNA for sodium channel, peroxidase, BMP-4 gene, bone morphogenetic protein.

In test group of 8d, similar sequences were: Ig variable region, DNA for mouse Ig, DNA for flexible peptide, tsr glkA, proteinase-3 neutroactin, EWS gene, repA protein.

Eighteen new sequences

No.1 sequence (AF240164)

1	ccgaattccc	gggtcgaccc	acgcgtccgg	tgtatgttct	tgccaatccc	agcacagttc
60	tacaaagtaa	aatatttggc	agtgaattca	tattaacaga	ggaacaaga	aattaacacg
120	tttttgcttc	gctaattcac	agttctttta	atacgctaat	tctaaatcac	ctgttctgac
180	tttgacaggc	tacagacacc	tgtttggggt	aatattccac	agctaattat	tacatgagaa
240	attcagtttc	caacaaaaga	gtttctgtgt	gaaattgcca	ttgtagttaa	caaattaata
300	tcaactatat	acaaatacat	ttctgatgtc	ttaatttaaa	tacagctaat	atactactca
360	tctataacta	aagaatggta	tataaaaact	aagactgccc	tcttagtgtt	tgaattctgt
420	ctgtcacact	ttacaccatg	gttgcactga	tcaagtcaga	atgttcttcc	cagtagcagt
480	aatttactgt	aatactgtat	tttaaatggc	aggaaaagag	tatagaattt	tcaagtggct
540	ggaaaaattc	ctaactgtat	gttaaaaatg	actcaggaga	gttaaaaaag	aaaaatactg
600	acaagagaga	gaaaaaaaaa	aaaaaagggc	ggccgctcta	gaggatccaag	cttacgtacg
660	cgtgcatgcc	gacgt				

No.2 sequence (AF240165)

1	gtgcccgacg	tcgcatgctc	ccggccgcca	tggccgcggg	atttatatat	atatatatat
60	atatatatat	atatatatat	ataaatcact	agtgcggccg	cctgcaggtc	gaccatatgg
120	gagagctccc	aacgcgttgg	atgcatagct	tgagtattct	atagtgtcac	ctaaatagct

180 tggcgtaatc atggtcatag ctgtttcctg tgtgaaattg ttatccgctc acaattccac
240 acaacatacg agccggaagc ataaagtgta aagcctgggg tgcctaatga gtgagctaac
300 tcacattaat tgcgttgcgc tcactgcccg ctttccagtc gggaaacctg tcgtgccagc
360 tgcattaatg aatcggccaa cgcgcgggga gaggcggttt gcgtattggg cgctcttccg
420 cttcctcgct cactgactcg ctgcgctcgg tcgttcggct gcggcgagcg gtatcagctc
480 actcaaaggc ggtaatacgg ttatccacag aatcagggga taacgcagga aagaacatgt
540 gagcaaaagg ccagcaaaag gccaggaacc gtaaaaaggc cgcgttgctg gcgtttttcc
600 ataggctccg ccccccctgac gagcatcaca aaaatcgacg ctcaagtcag aggtggcgaa
660 acccgacagg actataaaga tacccaggcg tttccccctg gaagctccct cgtgcgctct
720 cctgttccga ccctgccgct ttaccggata cctgtccgcc tttctcct

No.3 sequence (AF240166)
1 tgcaggtacc ggtccggaat tcccgggtcg acccacgcgt ccggcagtat atgacacaaa
60 tgttataatt agtgttattt cttttccaca ctgagtcaaa gattatttca cagctatttc
120 aacttttag atatcataat tctgaccata acactttaaa atattgtatt tattcattca
180 aaattcattg aaaaatcaca tgctattata ttaaaattat gtgattattt tacaaaatta
240 gacttacaga aaagaactat ttcctaccca aaaagctgta tgtttatatg cagcatgttt
300 tcaaaaaaaa aaaaaaaggg

No.4 sequence (AF240167)
1 tatgcggccc agccggccat ggcccaggtg aaactgcacc agtcaggacc tgaagtggta
60 aagcctgggg cttcagtgaa gctgtcctgc aaggcttcag gctacatctt cacaagttat
120 gatatagact gggtgaggca gacgcctgaa cagggacttg agtggattgg atggatttt
180 cctggagagg ggagtactga atacaatgag aagttcaagg gcagggccac actgagtgta
240 gacaagtcct ccagcacagc ctatatggag ctcactaggc tgacatctga ggactctgct
300 gtctatttct gtgctagagg ggactactat aggcgctact ttgacttgtg gggccaaggg
360 accacggtca ccgtctcctc aggtaccaag ctggagctga aacgggcggc cgcaggtgcg
420 ccggtgccgt atccggatcc gctggaaccg cgtgccgcat aga

No.5 sequence (AF240168)
1 ctttctatgc ggcccagccg gccatggccc aggtgaaact gcagcagtca ggacctgagg
60 tggtgaggcc tggggtctca gtgaagattt cctgcaaggg ttccggctac acattcactg
120 attattctat gcactggctg aagatgaatc atgcacagag tctagagtgg attggaatta
180 ttagtactta cgatggtaat acaaactaca accagaagtt taagggcaag gccactatga
240 ctgttgacaa atcctccatt acagcctata tggaacttgc cagattgaca tctgatgatt
300 ctgccatcta ttactgtgca agaggggctt actacggtag tttttattac tttgactact
360 ggggccaagg gaccacggtc accgtctcct caggtggagg cggttcaggc ggaggtggct
420 ctggcggtgg cggatcggaa tcgagctcac caggggcac caagctggaa atcaaacggg
480 cggccgcagg tgcgccggtg ccgtatccgg atccgctgga accgcgtgcc gcatagactg

No.6 sequence (AF240169)
1 ttgcggccca gccggccatg gcccaggtga aactgcagca gtcaggacct gaactgaaga
60 agcctggaga gacagtcagg atctcctgca aggcttctgg atataccttc acaactgctg
120 gaatgcagtg ggtgcaaaag atgccaggaa agggtttgaa gtggattggc tggataaaca
180 cccactctgg agtgccaaag tatgcagaag agttcaaggg acgctttgcc ttctctttgg
240 aaacctctgc cagcactgca tatttacaga taagcaacct caaaaatgag gacacggcta
300 cgtatttctg tatgagatgg gattacgacg gggggtttgc ttactggggc caagggacca
360 cggtcaccgt ctcctcaggt ggaggcggtt caggcggagg tggctctggc agtggcggat
420 cggacatcgt gctcacccag tctccagctt ctttggctgt gtctctaggg cagagggcca
480 ccatctcctg cagagccagc gaaagtgttg ataatattgg cattagtttt atgaactggt
540 tccagcagaa accaggacag ccacccaaac tcctcatcta tgctgcatcc aagcaaggat
600 ccggggtccc tgcaggttta ctggcaagtg ggtctgggac agatttcagc ctcaacatat
660 atcctatgga g

No.7 sequence(AF240170)
1 ccacgcgtcc ggcagtatat gacacaaatg ttatgattag tgttatttct tttccacact
60 gagtcaaaga ttatttcaca gctatttcaa ctttttagat atcataattc tgaccataac
120 actttaaaat attggattta ttcattcaaa attcattgaa aaatcacatg ctattatatt
180 aaaattatgt gattatttta caaaattaga cttacagaaa agaactattt cctacccaaa
240 aagctgtatg tttatatgca gcatgttttc aaaaaaaaaa aaaaagg

No.8 sequence(AF240171)
1 aacagtctat gcggcacgcg gttccagcgg atccggatac ggcaccggcg cacctgcggc
60 cgcccgtttg atttccagct tggtgccccc tggtgagctc gattccgatc cgccaccgcc

120 agagccacct ccgcctgaac cgcctccacc tgaggagacg gtgaccgtgg tcccttggcc
180 ccagtagtca aagtaataaa aactaccgta gtaagcccct cttgcacagt aatagatggc
240 agaatcatca gatgtcaatc tggcaagttc catataggct gtaatggagg atttgtcaac
300 agtcatagtg gccttgccct taaacttctg gttgtagttt gtattaccat cgtaagtact
360 aataattcca atccactcta gactctgtgc atgattcatc ttcagccagt gcatagaata
420 atcagtgaat gtgtagccgg aacccttgca ggaaatcttc actgagaccc caggcctcac
480 cacctcaggt cctgactgct gcagtttcac ctgggccatg gccggctggg ccgcatagaa
540 aggaacaact aaaggaattg cgaataataa ttttttcacg ttgaaaatct ccaaaaaaaa
600 ggcttcaaaa gcttggcgta atcatggtca tagctgnttn ctgtgtgaaa ttggtattcg
660 ctcacaattt cacacaat

No.9 sequence(AF240172)
1 tcgatagctg gtacgcctgc aggtaccggt ccggaattcc cgggtcgacc cacgcgtccg
60 gtgtatgttc ttgccaatcc cagcacagtt ctacaaagta aaatatttgg ccgtgaattc
120 atattaacag atggaacatg aaattaacac gtttttgctt cgctaattca cagttctttt
180 aatacgctaa ttctaaatca cctgttctga ctttgacagg ctacagacac ctgtttgggg
240 taatattcca cagctaatta ttacatgaga aattcagttt ccaacaaaag agtttctgtg
300 tgaaattgcc attgtggtta acaaattaat atcaactata tacaaataca tttctgatgt
360 cttaatttaa atacagctaa tatactactc atctataact aaagaatggt atataaaaac
420 taagactgtc ctcttagtgt ttgaattctg tctgtcacac tttacaccat ggttgcactg
480 atcaagtcag aatgttcttc ccagtagcag taatttactg taatactgta ttttaaatgg
540 caggaaaaga gtatagaatt ttcaagtggc tggaaaaatt cctaactgta tgttaaaaat
600 gactcaggag agttaaaaaa gaaaaattct gactggagag agaaaaaaaa gtagatgggc
660 ggggcgtttt agaaggatcc aagcttacgt ccgcgtgcat gccaaggcat aactcttcta
720 tcgggcacct aaattcaatt cctgggccgc ggttacacgg ccgggactgg ga

No.10 sequence(AF240173)
1 tctatgcggc acgcggttcc agcggatccg gatacggcac cggcgcacct gcggccgccc
60 gtttcagctc cagcttggtc ccccctccga acgtgtaagg aacctcctta ctttggtgac
120 agaaatacac tgcaggatca tcctcctcca taggatatat gttgaggctg aaatctgtcc
180 cagacccact gccagtaaac ctggcaggga ccccggatcc ttgcttggat gcagcataga
240 tgaggagttt gggtggctgt cctggtttct gctggaacca gttcataaaa ctaatgccaa
300 tattatcaac actttcgctg gctctgcagg agatggtggc cctctgccct agagacacag
360 ccaaagaagc tggagactgg gtgagcacga tgtccgatcc gccactgcca gagccacctc
420 cgcctgaacc gctccacctg aggagacggt gaccgtggtc ccttggccca gtaagcaaac
480 cccccgtcgt aatcccatct catacagaaa tacgtagccg tgtcctcatt ttttgaggtt
540 gcttatctgt aaatatgcaa gtgcctggca gaggtttcca aagagaaggc aaagccgtcc
600 ctttgaactc ttctggcata cttttggcac ttcagagtgg gtngtttatt ccagccaatc
660 cacttcaaaa ccctttttcct tgg

No.11 sequence (AF240174)
1 taccggtccg gaattcccgg gtcgacccac gcgtccggca gtatatgaca caaatgttat
60 gattagtgtt atttcttttc cacactgagt caaagattat ttcacagcta tttcaacttt
120 ttagatatca taattctgac cataacactt taaaatattg gatttattca ttcaaaattc
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240 cagaaaagaa ctatttccta cccaaaaagc tgtatgttta tatgcagcat gttttcaaaa
300 aaaaaaaaaa agggcggccg ctctagagga tccaagctta cgtacgcgtg catgcgacgt
360 catagctctt ctatagtgtc acctaaattc aattcactgg ccgtcgtttt acaacgtcgt
420 gactgggaaa accctggcgg ttacccaact taatcgcctt gcagcacatc ccccttтcgc
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540 gaatggcgaa tgggaccgcg ccctgtagcg gcgcattaag ccgcggcggg tgtggtggtt
600 acgcgcagcg tgaccgtaca cttggcagcg cccctatcgg ccgcttcctt tcgcttttct
660 tccctttcct tttttggcca cgttctgccc nggtttcccc gtcaagctct aaatccgggg
720 ggctcccttt taagggttcc gganttaang gttttaccgg nnccncgac cccg

No.12 sequence (AF240175)
1 cacagtaata gatggcagaa tcatcagatg tcaatctggc aagttccata taggctgtaa
60 tggaggattt gtcaacagtc atagtggcct tgcccttaaa cttctggttg tagtttgtat
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180 gccagtgcat agaataatca gtgaatgtgt agccggaacc cttgcaggaa atcttcactg
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300 gctgggccgc atagaaagga acaactaaag gaattgcgaa taataatttt ttcacgttga
360 aaatctccaa aaaaaaggct ccaaagcttg gcgtaatcat ggtcatagct gtttcctgtg
420 tgaaattgtt atccgctcac aattccacac aacatacgag ccggaagcat aaagtgtaaa

480 gcctggggtg cctaatgagt gagctaactc acattaattg cgttgcgctc actgcccgct
540 ttccagtcgg gaaacctgtc gtgccagctg cattaatgaa tcggncaacg cgccggggag
600 aggcggtttg cgtatt

No.13 sequence (AF240176)
1 tggaacattt tatttaaatg tcttgtgttc ccttttaacc aacaccaaaa agagaaatta
60 aaatttttt cttttttttc tttctttttt tttttgctat tccaaacagg ggagtcgctt
120 cagtgaaggg ttggcgagtc tctggagctg ggtggggagt gtgtgcgtcc tgtccggctg
180 ggttgttctt cccagcctgg ccaccctggg tagacagcca accccggagg tggcttcctg
240 ggagtctcac cctgagcaga cctggtcttc cctccaggga gggttgggtt gagggactgg
300 ctgtgactat gggacccagt gttccagaga gaaggggtag gggagagaag gtcagatctg
360 gaatgttcca tgatggttgc agggtctgga gagtatattt ggtagaaaaa taaggtgctt
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600 gttgattgtg ggtcgggtca gttggcatgg cacaaaaggg gagggggagc cggtttgtcc
660 atttgtgggc tttttgtggg tacctggcgg ctgcaaaaac atggttgccg tgtgggggaa
720 caccaaccca atggaaatca atcggggccc ccttggggag gggcttggtc ttagttac

No.14 sequence (AF240177)
1 ggcgaccgtg gtcccttggc cccagtaagc aaaccccccg tcgtaatccc atctcataca
60 gaaatacgta gccgtgtcct cattttgag gttgcttatc tgtaaatatg cagtgctggc
120 agaggtttcc aaagagaagg caaagcgtcc cttgaactct tctgcatact ttggcactcc
180 agagtgggtg tttatccagc caatccactt caaacccttt cctggcatct tttgcaccca
240 ctgcattcca gcagttgtga aggtatatcc agaagccttg caggagatcc tgactgtctc
300 tccaggcttc ttcagttcag gtcctgactg ctgcagtttc acctgggcca tggccggctg
360 ggccgcatag aaaggaacaa ctaaaggaat tgcgaataat aatttttca cgttgaaaat
420 ctccaaaaaa aaggctccaa agcttggcgt aatcatggtc atagctgttt cctgtgtgaa
480 attgttatcc gctcacaatt ccacacaaca tacgagccgg aagcataaag tgtaaagcct
540 ggggtgccta atgagtgagc taactcacat taattgcgtt gcgctcactg cccgctttcc
600 agtcgggaaa cctgtcgtgc cagctgcatt aatgaatcgg ccaacgcgcg gggagaggcg
660 gtttgcgtat tgggcgctct tc

No.15 sequence (AF240178)
1 aatctgaacg attgggcccg acgtcgcatg ctcccggccg ccatggcccg cgggatttac
60 ggctgcgaga agacgacaga attttttttt ttttttttt ttttttttt ggagaggggg
120 gtttctctat agccccggct gtcctggaac tcactatgta gaccaggctg gcttccagct
180 cacagaaatg cttttgcctc tgcctcctga gtgctggggt taagggactc tgcgttgtta
240 ccactgctta atcactagtg cggccgcctg caggtcgacc atatgggaga gctcccaacg
300 cgttggatgc atagcttgag tattctatag tgtcacctaa atagcttggc gtaatcatgg
360 tcatagctgt ttcctgtgtg aaattgttat ccgctcacaa ttccacacaa catacgagcc
420 ggaagcataa agtgtaaagc ctggggtgcc taatgagtga gctaactcac attaattgcg
480 ttgcgctcac tgcccgcttt ccagtcggga aacctgtcgt gccagctgca ttaatgaatc
540 ggccaacgcg cggggagagg cggtttgcgt attgggcgct cttccgcttc g

No.16 sequence (AF240179)
1 ccggtcccga cgtcgctgct cccggccgcc atggccgcgg gattatatat atatatatat
60 atatatatat atatatataa atcactagtg cggccgcctg caggtcgacc atatgggaga
120 gctcccaacg cgttggatgc atagcttgag tattctatag tgtcacctaa atagcttggc
180 gtaatcatgg tcatagctgt ttcctgtgtg aaattgttat ccgctcacaa ttccacacaa
240 catacgagcc ggaagcataa agtgtaaagc ctggggtgcc taatgagtga gctaactcac
300 attaattgcg ttgcgctcac tgcccgcttt ccagtcggga aacctgtcgt gccagctgca
360 ttaatgaatc ggccaacgcg cggggagagg cggtttgcgt attgggcgct cttccgcttc
420 ctcgctcact gactcgctgc gctcggtcgt tcggctgcgg cgagcggtat cagctcactc
480 aaaggcggta atacggttat ccacagaatc aggggataac cgcaggaaag aacatgtgag
540 caaaaggcca gcaaaaggcc aggaaccgta aaaaggccgc gttgctggcg tttttccata
600 gctccgcccc cctgacgagc atcacaaaaa tccgacgctc aagtcagagg tggcgaaacc
660 cgacaggact ataaagatac caagcgtttc cccctggaag ctccctcgtg cgctctcctg
720 ttccgaccct gccgcttacc ggatacctgg nccgcttttc tc

No.17 sequence (AF240180)
1 ctggttcgcc tgcaggtacc ggtccggaat tcccgggtcg acccacgcgt ccgcggacgc
60 gtgggcggac gcgtgggaaa agattgtgaa gcctgtgaaa gtttcagctc cccgagttgg
120 tggaaaacgc taaactggca gattagattt ttaaataaag attggattat aactctaaaa

180	aaaaaaaaaa	aagggcggcc	gctctagagg	atccaagctt	acgtacgcgt	gcatgcgacg
240	tcatagctct	tctatagtgt	cacctaaatt	caattcactg	gccgtcgttt	tacaacgtcg
300	tgactgggaa	aaccctggcg	ttacccaact	taatcgcctt	gcagcacatc	ccccttcgc
360	cagctggcgt	aatagcgaag	aggcccgcac	cgatcgccct	tcccaacagt	tgcgcagcct
420	gaatggcgaa	tgggacgcgc	cctgtagcgg	cgcattaagc	gcggcgggtg	tggtggttac
480	gcgcagcgtg	accgctacac	ttgcagcgcc	ctagcgcccg	ctcctttcgc	tttcttccct
540	tcctttctcg	ccacgttcgc	cggctttccc	cgtcaagctc	taaatcgggg	ggctcccttt
600	tagggtccga	tttagtgctt	tacggcacct	cgaccccaaa	aaaacttgat	tagggntgat
660	ggttcacgta	attgggccat	cgcccttgat	agacggtttt	ttcgcccttt	gacggttgga
720	agtccccgtt	tcttaataag	nggactnctt	gttccaaacn	tggaacaaca	cgtt

No.18 sequence (AF240181)

1	gacgtcgcat	gctcccggcc	gccatggccc	gcgggatatc	actagtgcgg	ccgcctgcag
60	gtcgaccata	tgggagagct	cccaacgcgt	tggatgcata	gcttgagtat	tctatagtgt
120	cacctaaata	gcttggcgta	atcatggtca	tagctgtttc	ctgtgtgaaa	ttgttatccg
180	ctcacaattc	cacacaacat	acgagccgga	agcataaagt	gtaaagcctg	gggtgcctaa
240	tgagtgagct	aactcacatt	aattgcgttg	cgctcactgc	ccgctttcca	gtcgggaaac
300	ctgtcgtgcc	agctgcatta	atgaatcggc	caacgcgcgg	ggagaggcgg	tttgcgtatt
360	gggcgctctt	ccgcttcctc	gctcactgac	tcgctgcgct	cggtcgttcg	gctgcggcga
420	gcggtatcag	ctcactcaaa	ggcggtaata	cggttatcca	cagaatcagg	ggataacgca
480	ggaaagaaca	tgtgagcaaa	aggccagcaa	aggccagga	accgtaaaaa	ggccgcgttg
540	ctggcgtttt	tccataggct	ccgccccccct	gacgagcatc	acaaaaattt	gctggcgttt
600	ttccataggc	tccgcccccc	tgacgagcat	cat		

DISCUSSION

After exposing to large dose ionizing radiation, intestinal crypt radiation death occurs, and no effective therapeutic measures are available to combat it. Data showed that the devastation or death of the crypt after irradiation is the crucial factor responsible for the pathogenesis. We have performed a series of experiments intending to increase the crypt survival after-irradiation in mice and confirmed that the nucleic acids (DNA, RNA) and their precursors may be used as one of the measures for the treatment of intestinal radiation syndrome that may occur in the war as well as in the peaceful uses of atomic energy[1-5]. However, the concrete molecular and cellular mechanisms are unknown.

Human genome group comprised 100 thousand of genes, which are selectively expressed, and determined the whole life course of organism, alteration of gene expressed levels is positioned at the centrel of controlling biological adjust mechanism[8]. Therefore, we think, after irradiation, between test group treated by RNA and the control group treated by physiological saline must exist differently expressed genes, which indicate that those genes were closely associated with intestinal crypt damage and repair. To isolate and clone these genes may not only be helpful to clarify the molecular mechanism of nuclear acids treatment, but also provide important basic theory for gene therapy of irradiation damage.

In the study, using BALB/c mice as studying target, we obtained 90 of genes a ssociated with repair of irradiation damaged intestinal gland cells. Data confirmed that hsp was increased at mRNA level after chronic radiation, PARP, serine protease-like gene, *p*53, *bcl*-2, bax, argainase I, ihsr PB7, Cdx1, NPT, PCNA, D1b-1, c-Ha-ras, c-myc, c-fos and so on were also increased at mRNA levels, which were correlated closely with drug treatment of irradiation damaged intestinal cells[9-25]. In our experiment, such as Nmi mRNA, Dutt1 protein, mRNA for Na, K-ATPase gamma subunit, mRNA for surface glycoprotein, Zinc finger type transcript factor, porcine growth hormone-releasing hormone gene, monocyte/ macrophage Ig-related gene, telomerase-associated protein, HOX1b protein, arginine/serine kinase, alkaline phosphatase mRNA, alkaline phosphatase 2, glkA gene *et al* were also closely correlated with repair of irradiation damaged intestinal crypt, what especially interesting was that RSG5 and ODC were identical to obtained sequences, data showed that RSG5, and ODC were overexpressed in irradiation-damaged intestinal crypt, and played an essential and positive role during DNA damage recovery and survival[26,27], our results also fully supported the conclusion. Although their concrete mechanism is not clarified, they may increase protein products by means of increased transcript levels to improve repair of irradiation-damaged intestinal crypt, and to suppress apoptosis of crypt cells[32].

Langberg *et al*[28] confirmed that immunological factors participated in the course of repair of irradiation damaged intestinal crypt such as IL-1, TGF-beta1, PDGF-AA, c-EGFR, EGF, TGF-beta-3. In our experiment, anti-CEA ScFv antibody gene, anti-DNA heavy chain, mRNA for Ig kappa chain, anti-BONT/A Hc ScFv antibody gene, mRNA for ScFv collagenase, AE0199 immunoglobulin heavy chain, mouse Ig gamma-chain, Ig rearranged gamma-chain mRNA, anti-c-myc antibody gene, anti-CD30 mAb ki-4 ScFv, anti-BSA antibody gene, D1 heavy chain, epidermal growth factor, anti-NP antibody IgH, mouse Ig

gammachain and haptoglobin were likely to be correlated closely with repair of irradiation damaged intestinal crypt. What is especially interesting is several gene fragments were partly identical to sequences of ScFv genes, this point was not able to be expressed clearly. Our results support that immunological factors exert effect on the course of repair of irradiation damaged intestinal crypt[29-45].

In our experiment, eighteen novel sequences were obtained, their concrete fun ctions are still unclear. But we believe that these genes are closely associated with irradiation treatment, only if we clarify the function of these genes, and according to the changes of these genes, to design a controlling measure, we are likely to decrease irradiation damage, and also provide new thoughts for tumor radiation treatment[46-64].

In summary, our results primarily demonstrate that nuclear acids are capable of improving repair of irradiation damaged inte stinal crypt, its action may be closely correlated with increased mRNA levels of some genes, also with immun ological factors, but the concrete molecular mechanism such as signal transduc tion and suppression of apoptosis still needs further studies[65-89].

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