研究报告

外源核酸促核辐射鼠肠腺细胞修复的基因分析*

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摘要 初步探讨外源核酸促电离辐射损伤的小鼠肠腺细胞修复的分子机理. 建立 BALB/c 小鼠电离辐射后给予外源 RNA 与生理盐水治疗的6 h、12 h、24 h、4 d 和 8 d 的模型，采集空肠组织标本后采用消减杂交基础上的 LD-PCR 技术，获取与受照小鼠肠腺损伤修复相关的基因克隆，对其进行全自动序列分析与 GenBank 检索. 6 h 治疗组同源性较高的序列主要为：热休克蛋白 mRNA、Nmi mRNA、Dutt1 蛋白 mRNA、Na, K-ATPase γ 亚单位 mRNA 等；12 h 治疗组同源性较高的序列为：碱性磷酸酶 mRNA、碱性磷酸酶 2、glkA 基因、单链复制着丝粒基因等；24 h 治疗组同源性较高的序列为：抗 CEA 单链抗体重链可变区基因、抗 DNA 重链可变区基因、Ig kappa 链 mRNA 等；4 d 治疗组同源性较高的序列为：双特异性磷酸酶、端粒酶相关蛋白家族 mRNA、β-GABA 转运基因、紧张激活蛋白 mRNA、FK506 结合蛋白、Ca^{2+} / Ca^{2+} 调蛋白依赖性基因等；8 d 治疗组同源性较高的序列为：免疫球蛋白可变区基因、鼠免疫球蛋白 DNA、易弯曲肽 DNA、tsr glkA 基因、修复蛋白 A 等. 新发现的 18 个基因片段递交给 GeneBank，接受号为 AF240164-AF240181. 结果表明：外源核酸促电离辐射损伤的小鼠肠腺修复的机理可能与一些基因、蛋白质的异常表达有关，与免疫系统的作用可能有关.

关键词 小鼠，电离辐射，修复，表达基因，机理
学科分类号 R823.7

核爆炸和核事故条件下大剂量电离辐射引起肠腺细胞的死亡是肠道辐射损伤的关键，严重时可发生肠型放射病而引起死亡. 从 60 年代起，国内外进行了多项研究，但至今仍未见有效的治疗措施报道. 为了增强肠腺细胞的存活率，我们自 70 年代起就开展了如何提高受照射小鼠肠腺细胞存活与机理的研究. 实验证实正常小鼠肠腺细胞悬液和多种组织器官粗提液中含有提高受照小鼠肠腺存活的因子，并证实此因子的化学成分为核酸 (DNA、RNA) 及其前体，进一步实验证实外源核酸对受照小鼠肠腺的修复是它在体内的酶解产物 (包括单独一种单核苷酸、核苷和碱基) 作用所致[1~7]. 但是，核酸及其前体促进电离辐射损伤的肠腺细胞修复的分子机理仍不清楚. 本课题首选建立 BALB/c 小鼠电离辐射后给予外源 RNA 与生理盐水治疗的模型，分别于治疗后6 h、12 h、24 h、4 d、8 d 采集空肠组织标本，采用消减杂交基础上的 Long Distance-PCR 技术[7]，克隆了外源核酸对受照小鼠肠腺损伤修复相关的表达基因，并对其进行了测序与检索分析，拟初步探讨外源核酸促电离辐射损伤的肠腺修复的分子机理.

1 材料与方法

1.1 材料

mRNA 提取采用 Promega 公司 PolyATract System 1000 试剂盒，反转录采用 SMART™ PCR cDNA Synthesis Kit (Clontech 公司)，PCR 产物纯化采用 Promega 公司 Wizard Plus Minipreps DNA Purification System，LD-PCRes et Advantage 2PCR Kit (Clontech)；PCR 扩增仪采用 PE-5700 型定量 PCR 仪，PGEM -T easy Vector System 购自 Promega 公司，α-^{32}pdATP 购自北京福瑞公司，化学试剂购自原平公司.

1.2 方法

按文献 [4] 的方法从 LACA 正常小鼠小肠中提取总 RNA. 取 90 只 BALB/c 雄性小鼠，10~ 12 周龄，体重 18~ 22 g，实验前于实验室内饲养 2 周以适应环境. 按文献 [7] 的方法进行随机分组、照射与治疗. 为了避免个体差异，实验组 6 h、

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12 h、24 h、4 d、8 d 的小肠标本，相同条件的混合在一起，mRNA 的提取、反转录、消减杂交、LD-PCR 按 Clone Tech 说明书进行，PCR 产物克隆入 T 载体、反向杂交鉴定按文献 [7] 进行. 质粒的提取采用 VIOGENE 公司的 plasmid DNA purification miniprep kit，质粒的定量采用紫外分析仪进行，测序采用 Model 377 型全自动序列分析仪进行测序分析. 测序结果借助联网计算机进行检索分析，新基因递交给 GenBank.

2 结　果

2.1 与 RNA 促肠腺修复的表达基因克隆结果

所获克隆用 BstZ1 酶切后证实含有相应片段 (图 1).

实验组 6 h、12 h、24 h，4 d 和 8 d 的克隆表达基因数分别为 18、22、25、23、12 个，编号依次为 XCZ1~ 90，即获取了 90 个与损伤肠腺修复相关的基因克隆.

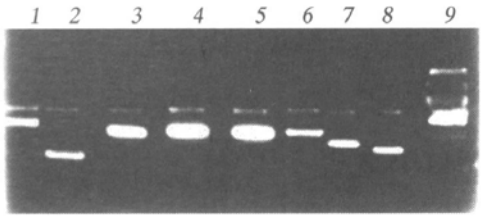

Fig. 1 Part result of cloned plasmids cut by BstZ1 enzyme

1~ *8*: results of products cut by BstZ1; *9*: Marker.

2.2 杂交鉴定结果

所获基因在实验组与对照组之间呈差异表达 (图 2).

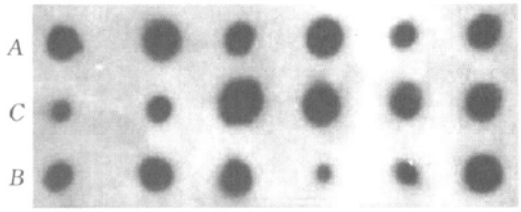

Fig. 2 Part results of Northern hybridization

A: result of hybridization with RNA from test group; *B*: result of hybridization with RNA from control group; *C*: result of hydridization with RNA from normal group.

2.3 测序与联机检索分析结果

所获的 90 个克隆全部测序成功. 对所获序列进行了 GenBank 检索，不同时间点的序列主要检索分析结果如下:

实验组 6h 同源性较高的序列见表 1.

Table 1 Sequences in test Group of 6 h higher similar to those in GenBank

Clone number	Similar gene	GenBank number
XCZ1	mRNA for heat shock protein	AJ238010.1
XCZ2	Nmi mRNA	AF019249
XCZ3	Dutt1 protein	Y17793
XCZ4	mRNA for Na, K-ATPase gamma subunit	Y11587
XCZ5	mRNA for surface glycoprotein	Z50022
XCZ6	Zinc finger type transcript factor	AF082568
XCZ7	porcine growth hormone-releasing hormone gene	L11869
XCZ8	Homo sapiens dual specificity phosphatase	NM_007207.1
XCZ9	monocyte/macrophage Ig-related gene	AF004231
XCZ10	telomerase-associated protein	NM-007110.1
XCZ11	mRNA for arginine/serine kinase	X99437
XCZ12	porcine growth hormone-releasing hormone	L11869

实验组 12 h 同源性较高的序列见表 2.

Table 2 Sequences in test group of 12 h higher similar to those in GenBank

Clone number	Similar gene	GenBank number
XCZ19	alkaline phosphatase mRNA	J03572
XCZ20	alkaline phosphatase 2	NM-007431.1
XCZ21	glkA gene	X98363
XCZ22	single stranded replicative centromeric gene	X70272
XCZ23	Homo sapiens DMBT1 candidate tumor gene	AJ243211.1
XCZ24	tRNA-Met gene	AF059172
XCZ25	Mouse Ig unrearranged transcribed H-chain	M13787
XCZ26	thyroxine-binding globulin gene	L13470
XCZ27	alpha-2-plasmin inhibitor gene	M20782
XCZ28	mus musculus tight-skin strain autoantibody	U09597
XCZ29	M. domesticus IgG variable region	Z22059
XCZ30	Mus musculus rearranged immunoglobulin heavy chain	Z15019
XCZ31	Mouse Ig active heavy chain mRNA V-region	J00494
XCZ32	M. Musculus mRNA for IG heavy chain vary region	X63048
XCZ33	Mus musculus Ig active kappa-chain mRNA	M64160
XCZ34	Murine/human chimeric anti-idiotype antibody	X66205
XCZ35	Mouse Ig heavy chain VH region mRNA	L41808
XCZ36	M. musculus germline gene for immunoglobin	K02160

实验组 24 h 同源性较高的序列见表 3.

Table 3 Sequences in test group of 24 h higher similar to those in GenBank

Clone number	Similar gene	GenBank number
XCZ41	anti-CEA ScFv antibody heavy chain vary region	AB001737
XCZ42	anti-DNA antibody Ig heavy chain	U30234
XCZ43	mRNA for Ig kappa chain region	U62633
XCZ44	anti BONT/A Hc ScFv antibody heavy chain vary region	AF003722
XCZ45	mRNA for ScFv collagenase heavy chain vary region	AB001738
XCZ46	AE0199 immunoglobulin heavy chain	AF118966.1
XCZ47	Mouse Ig gamma-chain	M34523
XCZ48	Ig rearranged gamma-chain mRNA	L14358
XCZ49	anti-c-myc antibody	AJ000489
XCZ50	anti-CD30 antibody	AF002242
XCZ51	Mus musculus A-kinase anchoring protein	AF047716
XCZ52	anti-BSA antibody D1 heavy chain	AF083188
XCZ53	Homebox protein Xgbx-2 mRNA	U04867
XCZ54	epidermal growth factor	U76382
XCZ55	anti-NP antibody IgH	M60249
XCZ56	mRNA for arginine/serine kinase	X99437
XCZ57	dual specificity phosphatase	NM-007207.1
XCZ58	family mRNA telomerase-associated protein	NM_007110.1
XCZ59	anti-human erbB-2 region	U64994
XCZ60	BMP-4 geneA	J005076

实验组 4 d 同源性较高的序列见表 4.

Table 4 Sequences in test group of 4d higher similar to those in GenBank

Clone number	Similar gene	GenBank number
XCZ66	unguis-cati Acyl-ACP desaturase	AF051134
XCZ67	Cavia porcellus mRNA for sodium channel	AJ249296.1
XCZ68	bone morphogenetic protein	AF058764
XCZ69	tazarotene-induced gene2	U77594
XCZ70	betaine-GABA transporter gene	U27699
XCZ71	homobox protein Xgbx-2 mRNA	UO4867
XCZ72	mRNA for stress-activated protein	Y15075
XCZ73	FK506 binding protein	AF090334
XCZ74	calium/calmodulin dependent gene	X77933
XCZ75	PEST phosphatase interactin gene	U87814
XCZ76	haptoglobin mRNA	L10353

实验组 8 d 同源性较高的序列见表 5.

Table 5 Sequences in test group of 8d higher similar to those in GenBank

Clone number	Similar gene	GenBank number
XCZ79	Ig Mu variable region mRNA	AF015482
XCZ80	Mus musculus IgK chain mRNA V-region	M83099
XCZ81	DNA for flexible peptide	D50400
XCZ82	tsr glkA	X98363
XCZ83	mRNA for Hox1b protein	X92428
XCZ84	Mus musculus neutroactin mRNA	AF010586
XCZ85	Rat alkaline phosphatase mRNA	J03572
XCZ86	Human mRNA for XP-C repair complementing protein	D21090.1
XCZ87	Human alpha-2-plasmin inhibitor gene	M20782
XCZ88	mRNA for CCAAT binding factor	Z70024
XCZ89	Mouse active H-chain VJ region	M74138

2.4 新基因序列分析结果

GenBank 分析表明：90 个序列中，18 个是与肠腺的损伤修复密切相关的新序列，GenBank 接受号为 AF240164～AF240181. 实验组 6 h、12 h、24 h、4 d 和 8 d 的新表达基因数分别为 4、4、5、3、2 个.

3 讨 论

消化道是电离辐射敏感组织，其中尤以小肠最为敏感. 多年来，国内外对电离辐射后肠上皮干细胞的损伤、肠道组织形态和消化吸收功能的改变作了较多的研究[1~7]，但如何促进辐射损伤的肠腺细胞修复的报道较少. 我们的大量实验表明外源核酸对辐射损伤的肠腺具有较好的修复治疗作用[1~8]，其机理不清楚. 本课题组拟从表达基因水平，来探讨核酸促辐射损伤的肠腺修复的分子机理.

本实验对获取的外源 RNA 促受照小鼠肠腺损伤修复的相关表达基因 90 个，即实验组 6 h、12 h、24 h、4 d 和 8 d 的基因克隆数分别为 18、22、25、13、12 个，对所获基因克隆进行了双向测序，GenBank 检索分析.

从检索分析结果可以看出：核酸治疗肠腺细胞损伤时，在 6 h 时可引起热休克蛋白、锌指型转录因子、甲状腺结合球蛋白、碱性磷酸酶基因、损伤修复相关基因等高表达；随着时间的延长，在 12 h 时，出现了免疫相关基因如 Mus musculus Ig active

kappa-chain mRNA 等高表达，在 24 h、4 d、8 d 皆出现免疫相关基因高表达，这表明免疫系统可能在损伤后 12 h 开始参与肠腺细胞的修复过程，并参与以后的修复过程. Langberg 等[9] 证实辐射损伤的肠腺在修复过程中存在免疫因子 IL-1、TGF-beta1、PDGF-AA、c-EGFR、EGF、TGF-beta-3 差异表达，也支持免疫因素在肠腺修复过程中起一定的作用. 进一步分析提示：外源 RNA 促肠腺的修复时，还存在 DNA 聚合酶基因、肌胞浆磷酸蛋白、Na，K-ATP 酶等基因高表达，还存在单链复制的着丝粒基因高表达，特别有意义的是还存在端粒酶相关蛋白质与基因高表达，这些基因涉及到离子通道、细胞分裂等过程. 有趣的是在核酸治疗 6 h组开始出现碱性磷酸酶基因高表达，并持续到 8 d，这些碱性磷酸酶在损伤修复过程中起何作用，有待于进一步探讨.

目前文献 [10~18] 显示：与辐射损伤修复相关基因有 PARP，serine protease like gene，p53，bcl-2，bax，argainase Ⅰ，ihsrPB7，Cdx1，NPT，PCNA，D1br1，c-Ha-ras，c-myc，c-fos，RSG5，ODC. 本实验结果与其中部分报道一致，另外，还有一些参与修复基因未见报道，如 homebox，BMP-4 等[19,20]. 另外，在本实验中，我们还获取了 18 个新序列，它们的具体功能还不清楚，但可能与辐射损伤的肠腺修复有关. 我们相信：只有阐明这些基因的具体功能，设计调控措施，有可能使辐射对肠腺的损伤减少到最低程度.

总之，我们认为，外源核酸促进辐射损伤肠道的修复作用可能与免疫系统、碱性磷酸酶、抗 CEA 抗体、转录因子、修复基因、分子伴侣等密切相关，还与一些未知功能的基因有关. 但是，这些基因与蛋白质是如何进行修复，如何抑制凋亡，其信号转导过程如何，仍有待于进一步研究.

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Analysis of Genes Associated with Exogenous Nucleic Acids Improving the Repair of Intestinal Epithelium After γ Irradiation in Mice*

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Abstract In order to explore the molecular mechanism of exogenous nucleic acids improving repair of irradiation-damaged intestinal epithelium, 45 mice being irradiated by γ ray were treated with 40 μg small intestinal RNA as test group, whose small intestinal specimens were collected respectively at 6 h, 12 h, 24 h, 4 d and 8 d after treatment; 40 mice being irradiated by γ ray were treated with physiological saline as control group, whose small intestinal specimens were collected at the same interval time. Then fragments of genes expressed in test group higher than those in control group, were obtained by using LD-PCR based on subtractive hybridization. After that, these gene fragments were cloned into T vectors, and were sequenced. Obtained sequences were searched for GenBank. 90 clones associated with repair of irradiation-damaged crypt cells were obtained. In test group of 6 h, higher 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, Homo sapiens dual specificity phosphatase, etc. In test group of 12 h, higher similar sequences were as follows: alkaline phosphatase mRNA, alkaline phosphatase 2, glkA gene, single stranded replicative centromeric gene, Homo sapiens DMBT1 candidate tumor gene, tRNA-Met gene, mouse Ig unrearranged transcribed H-chain, thyroxine-binding globulin gene, alpha-2-plasmin inhibitor gene, etc; In test group of 24 h, higher similar sequences were as follows: anti-CEA ScFv antibody heavy chain vary region, anti-DNA antibody Ig heavy chain, mRNA for Ig kappa chain region, anti-BONT/A Hc ScFv antibody heavy chain vary region, mRNA for ScFv collagenase heavy chain vary region, AE0199 immunoglobulin heavy chain, mouse Ig gamma-chain, Ig rearranged gamma-chain mRNA, anti-NP antibody IgH, mRNA for arginine/serine kinase, dual specificity phosphatase, family mRNA telomerase-associated protein, anti-human erB-2 region, BMP-4 gene, etc; In test group of 4 d, higher similar sequences were as follows: mRNA for sodium channel, tazarotene-induced gene, betaine-GABA transporter gene, homobox protein Xgbx-2 mRNA, mRNA for stress-activated protein, FK506 binding protein, calium/calmodulin dependent gene, PEST phosphatase interactin gene, haptoglobin mRNA, etc; In test group of 8 d, higher similar sequences were as follows: Ig Mu variable region mRNA, Mus musculus Ig K chain mRNA V-region, mRNA for Hox1b protein, Mus musculus neutroactin mRNA, rat alkaline phosphatase mRNA, Human mRNA for XP-C repair complementing protein, human alpha-2-plasmin inhibitor gene, mRNA for CCAT binding factor, mouse active H-chain VJ region, etc. Eighteen were new sequences, whose function were unclear. Ninety clones were obtained to be associated with repair of damaged mice intestinal gland cells caused by γ ray and treated by small intestinal RNA. Repair of damaged intestinal gland cells treated by exogenous nucleic acids may be associated with hsp, Nmi, Dutt1, alkaline phosphatase genes and eighteen new sequences.

Key words mouse, ionizing irradiation, repair, expression gene, mechanism

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