

小剂量辐射诱导小鼠免疫细胞早期 蛋白质合成动力学变化

陈沙力 刘树铮

(白求恩医科大学卫生部放射生物实验室 长春 130021)

摘要 采用 ^3H -Leu 掺入示踪观察 75 mGy X 射线全身照射对小鼠免疫细胞蛋白质早期合成的影响。结果表明,小剂量 X 射线全身照射后小鼠胸腺细胞蛋白质合成开始增加,6~8h 达到峰值,随后逐渐下降。SDS-PAGE 及光密度扫描结果表明,照射后小鼠胸腺及脾细胞蛋白质合成增加,同一细胞中不同分子量的蛋白质各有其消长特点,不同细胞间合成增加的蛋白质分子量有所不同。这些早期变化的蛋白分子参与复杂的细胞功能调节,可能与辐射兴奋效应有关。

关键词 小剂量辐射,免疫细胞,蛋白质合成动力学

小剂量电离辐射诱导激活的基因产物可能参与适应性反应、免疫增强、肿瘤抑制及神经内分泌调节等过程^[1,2]。虽然确切的发生机制还不清楚,但进一步地深入研究无疑将帮助我们了解辐射诱导新蛋白质的本质和功能,对揭示辐射兴奋效应的分子机制具有重要意义。早先的研究表明,小剂量全身照射可诱导小鼠胸腺细胞数种蛋白分子变化,包括新蛋白质、增强蛋白质、减弱蛋白质及消失蛋白质^[3]。为了进一步了解小剂量辐射对免疫细胞早期蛋白质合成的影响,本研究对照射后胸腺及脾细胞蛋白质合成动力学变化进行了观察。

1 材料和方法

1.1 动物及照射条件

健康雄性昆明小鼠,体重 18~22g。采用 Philips 深部 X 射线机照射动物,电压 200 kV,电流 10 mA,滤板 0.5 mmCu 和 1.0 mm Al,靶皮距离 212 cm。单次全身照射,剂量率为 12.5 mGy/min,剂量为 75mGy。

1.2 小鼠胸腺及脾细胞悬液的制备

于照射后 0、2、4、6、8 及 12 h 分别断头处死小鼠,取胸腺及脾分别置 RPMI 1640 培养液中,毛玻片挤压,制成单细胞悬液,用 200 目尼龙网过滤,去除结缔组织,在 1000rpm 下离心 5min,沉淀细胞用 PBS 洗涤 2 次,脾细胞加 10 倍体积的红细胞裂解液, PBS 洗涤 2 次。同时制备正常细胞悬液,细胞与 ConA ($5\mu\text{g}/\text{ml}$) 反应 0~12h 作为对照。

1.3 ^3H -Leu 代谢掺入示踪

用含 10% NBS、2mmol/L 谷氨酰胺,0.05mmol/L 二巯基乙醇的无亮氨酸 RPMI 1640 培养液将胸腺细胞浓度调至 $2.5 \times 10^6/\text{ml}$,在 96 孔培养板上每孔加 200 μl 细胞悬液, ^3H -Leu 18.5 kBq (比活性 5×10^4 kBq/mg),设三复孔在 37°C,5% CO₂ 孵箱中分别培养 4、6、8 和

12 h。试验组为 75 mGy X 射线全身照射后 4 h 所分离的胸腺细胞，对照组为假照射小鼠胸腺细胞。收集细胞后用液闪计数器测定 (cpm)，以 $^3\text{H}\text{-Leu}$ 掺入量估算蛋白质合成速率。

1.4 蛋白质提取

预冷经 PBS 洗涤的细胞及裂解液 (50 mmol/L Tris pH8.0, 150 mmol/L NaCl, 1% NP-40, 0.3% SDS, 0.5 mmol/L PMSF) 至 0℃，每 10^7 个细胞加 1 ml 裂解液，冰浴 30 min，间或振荡，反复通过 22 号针头，4℃ 12000 g 离心 10 min，上清加等体积 2×SDS 电泳样品缓冲液，沸水浴 5 min，-70℃ 下保存备用。

1.5 SDS 聚丙烯酰胺凝胶电泳及光密度扫描

照射组、ConA 刺激组及对照组蛋白质提取物行 SDS 聚丙烯酰胺凝胶电泳 (SDS-PAGE)，10% 分离胶，胶高 12.5 cm，4.5% 浓缩胶，胶高 2.5 cm，蛋白质样品上样量为 150μg，100 V 电泳 6~8 h，考马斯亮蓝 R-250 染色，脱色后光密度扫描定量^[4]。

2 结果

2.1 $^3\text{H}\text{-Leu}$ 掺入胸腺细胞的早期蛋白质合成

75 mGy X 射线全身照射小鼠后 4 h 取胸腺，细胞分别培养 4、6、8 和 12 h， $^3\text{H}\text{-Leu}$ 掺入示踪蛋白质合成的变化，结果表明 (见图 1)，照射组细胞蛋白质合成明显增加，与对照组细胞比较依时间顺序分别增加 33.26%、51.48%、51.54% 和 34.98%。提示小剂量 X 射线全身照射后胸腺细胞蛋白质合成开始增加，6~8h 达高峰，随后逐渐下降。

2.2 不同分子量蛋白质合成的变化

采用 SDS-PAGE 及光密度扫描观察不同分子量蛋白质合成变化的结果表明，75mGy X-射线全身照射后胸腺细胞 28 kD 蛋白质合成在 12 h 内逐渐增加；43 kD 蛋白质合成在 2 h 达高峰，随后渐减，12 h 后趋于正常。而 ConA (5μg/ml) 刺激细胞 28 kD 蛋白质合成无明显变化，43 kD 蛋白质合成在 12 h 内逐渐增加，其增加幅度高于辐射诱导的 (见图 2)。75 mGy X 射线全身照射后脾细胞 43 kD 蛋白质合成在 2 h 后开始增加 (见图 3)，32 kD 蛋白质合成在 2 h 达到高峰，随后渐减，其增加幅度明显高于 ConA 刺激。ConA (5μg/ml) 刺激细胞 32 kD 蛋白质合成在 2 h 达到高峰，随后渐减，12 h 则低于正常水平，而 43 kD 蛋白质合成无明显变化。以上结果提示，小剂量 X 射线全身照射诱导小鼠免疫细胞蛋白质合成增加，同一细胞中不同分子量的蛋白质各有其消长特点，胸腺和脾细胞间合成增加的蛋白质分子量有所不同。

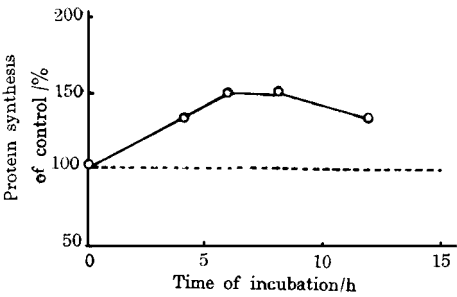


Fig.1 Effect of WBI with 75 mGy X-rays on protein synthesis of thymocytes in mice. Cells of sham-irradiated and X-irradiated mice were metabolically labelled for 4, 6, 8 and 12 h with $^3\text{H}\text{-Leu}$ 4 h after WBI with 75 mGy X-rays. $^3\text{H}\text{-Leu}$ incorporation was measured by scintillation counter as described in materials and methods

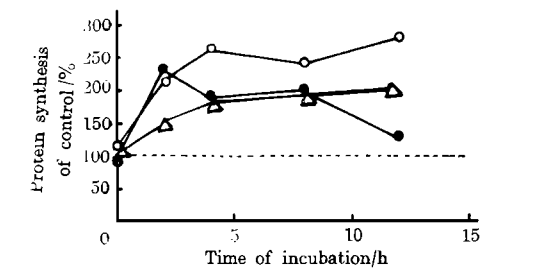


Fig. 2 Changes in protein synthesis of thymocytes in mice 0~12 hours after WBI with 75 mGy X-rays Thymytes of sham-irradiated and X-irradiated mice with or without Con A stimulation (5 μ g/ml) for 0~12 hours were lysed and protein extracts were run on a 10% SDS-PAGE gel. The gel was subsequently densitometrically analysed and relative increases in the 28 kD and 43 kD protein levels was quantified
($\triangle$) 75 mGy, 28 kD ($\bullet$) 75 mGy, 43 kD
($\circ$) Con A, 43kD

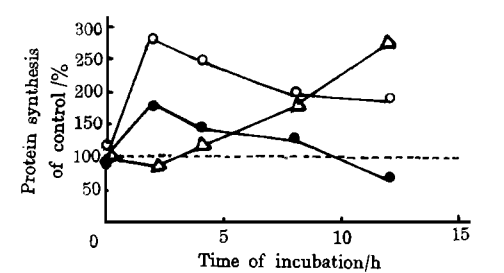


Fig. 3 Changes in protein synthesis of splenocytes in mice 0~12 hours after WBI with 75 mGy X-rays Splenocytes of sham-irradiated and X-irradiated mice with or without Con A stimulation (5 μ g/ml) for 0~12 hours were lysed and protein extracts were run on a 10% SDS-PAGE gel. The gel was subsequently densitometrically analysed and relative increases in the 28 kD and 43 kD protein levels was quantified
($\triangle$) 75 mGy, 28 kD ($\bullet$) 75 mGy, 43 kD ($\circ$) Con A, 43kD

3 小 结

已有大量实验研究和人体的观察资料证明，小剂量辐射作用于哺乳动物和人体主要可诱导适应性反应、增强免疫功能、提高抗癌能力，三者是彼此联系的^[5]。目前认为适应性反应的诱导与蛋白质表达和修复酶类激活有关，这涉及某些蛋白分子的合成，同时也涉及另一些蛋白分子的合成抑制^[6]。

研究表明 75 mGy X 射线全身照射后 4 h 胸腺细胞体外合成蛋白质增加，于培养的 6~8 h 达峰值，这与诱导适应性反应的间隔时间一致，提示适应性反应的诱导可能与某些蛋白质表达有关。照射后小鼠免疫细胞 28 kD、32 kD 和 43 kD 蛋白分子增强表达，可能作为保护性蛋白质发挥作用作用。虽然合成增加的蛋白质在胸腺及脾细胞中有所不同，但均具有酪氨酸磷酸化特性（资料未表明）。这些蛋白分子是否参与适应性反应等辐射兴奋效应机制值得进一步研究。

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DYNAMIC CHANGES OF THE EARLY PROTEIN SYNTHESIS IN MURINE IMMUNE CELLS AFTER LOW DOSE RADIATION

Chen Shali Liu Shuzheng

(MPH Radiobiology Research Unit, Norman Bethune University of Medical Sciences,
Changchun 130021)

ABSTRACT It was shown that there was a marked increase in protein synthesis of thymocytes that were metabolically labelled with ^3H -Leu for 4, 6, 8 and 12 hours in low dose irradiated mice showing 33.26%, 51.48%, 51.54% and 34.98% increase respectively at different time intervals of incubation when the thymic and splenic cells were sampled 4 hours after whole body irradiation (WBI) with 75 mGy X-rays. The results suggest that there is an increase in protein synthesis with its peak at 6~8 hours after radiation. Changes in protein synthesis of immune cells in mice 4 hours after radiation and incubated for 4~12 h were observed with SDS-PAGE followed by densitometrical scanning. It is revealed that 28 kD protein synthesis was increased gradually within 12 hours of incubation and 43 kD protein synthesis was increased in the thymocytes rapidly reaching a maximum 2 hours after incubation. It was also exhibited that the synthesis of 43 kD protein and 32 kD protein was increased in the splenocytes 2 hours after incubation. These findings may have implications in the mechanism of immunoenhancement and adaptive response induced by low dose radiation.

KEYWORDS Low dose radiation, Immune cell, Protein, Synthesis

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MECHANISM STUDY OF VASCULAR ENDOTHELIAL CELL RADIATIVE INJURY INFLUENCING B¹⁶ MELANOMA INVASION

Zhu Hanneng Shen Mingli

(Shanghai Institute of Radiation Medicine, Shanghai Medical University, Shanghai 200032)

ABSTRACT In order to test the influence of endothelial cell radiative injury on the tumor invasion rate at different time after exposure to $^{15}\text{Gy } ^{60}\text{Co } \gamma$ -ray, we cultured vascular endothelial cell in vitro and set up B¹⁶ melanoma invasion model. Meanwhile, the secretion of PGI₂, TXB₂, TPA and PAI from endothelial cells after irradiation were measured by radioimmunologic and enzyme labeling techniques. The results showed that for endothelial cells exposed to radiation the invasion rate of tumor cell were considerably larger than that of control, the secretion of PGI₂ was decreased significantly, the secretion of both TXB₂ and TPA were increased obviously. The phase change of invasion rate corresponds with the changes of TXB₂, PGI₂ and TPA. The results suggested that endothelial cells play an important role in tumor invasion and metastasis, the disorder of endothelial cell anticoagulation and fibrin lysis function caused by radiation might be the main reason for endothelial cell radiative injury accelerating the B¹⁶ melanoma invasion.

KEYWORDS Radiation injury, Vascular endothelial cell, B¹⁶ melanoma cell invasion