

快报

用流式细胞计数仪检测不同辐射剂量诱导的小鼠胸腺细胞程序性死亡

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关键词 程序性细胞死亡, 流式细胞术, 胸腺细胞, X射线

自 Wyllie 等^[1]对程序性细胞死亡(PCD), 又名细胞凋亡(apoptosis)的意义进行综述以来, 对其发生机制的研究越来越多。普遍认为这种细胞死亡在胚胎发育、细胞增殖与分化以及肿瘤生长等研究方面均有重要意义。Cohen 等^[2]对 PCD 在免疫系统中的作用进行了详细的评述。认为 PCD 的形态特点是核质浓缩, 核膜完整而胞体变小, 其分子标志是 DNA 裂解(fragmentation), 形成大小约 180 bp 或其倍数的断片, 其行为特征是因细胞表面分子改变而被邻近的巨噬细胞吞噬, 就地清除。因而它与另一种细胞死亡方式(坏死), 具有截然不同的性质。

检查 PCD 的方法很多, 包括细胞大小和比重, DNA 梯形图象, “慧星”检测, 细胞膜通透性等。本实验采用 Garvy 等人^[3]报道的流式细胞计数(FCM)检测法, 对小鼠受不同剂量 X 射线体外照射后胸腺细胞 PCD 的变化进行了研究。

按照文献[4]报道的方法, 取昆明小鼠胸腺制成单个细胞悬液, 加 RPMI-1640 培养液和 1×10^6 细胞于 24 孔培养板中。照射后, 于 5% CO₂, 37℃培养箱中培养。以 Propidium iodide (PI) (Sigma)为荧光探针标记细胞内的 DNA。由于在程序性死亡过程中, 核 DNA 裂解成寡聚核小体, 并在流式细胞计数仪(B-D FACScan)所获得的细胞周期直方图的 Ao 区, 形成一个峰(见图 1), 该峰可用于 PCD 定量分析。

未经照射的正常胸腺细胞, 在体外培养过程中, PCD 呈时间依赖性增高(见表 1)。2.0 Gy X 射线体外照射后 4、8、12 和 24 h, PCD 随时间明显增加, 并显著高于单倍培养组(见图 2)其中 4 h 时 $p < 0.05$, 8 h、12 h 和 24 h 时 $p < 0.01$ 。由表 2 可见, 胸腺细胞经不同剂量 X 射线体外照射后 24 h, 剂量小于 100 mGy, 与单倍培养组相比, 其 PCD 无明显差异。而当剂量大于 100 mGy 时可见 PCD 显著增高, 特别是小鼠胸腺细胞受中等致死剂量(6.0 Gy)照射后 24 h, PCD 达到 78.34%。

Tab 1. Changes in PCD of normal thymocytes cultured in vitro

Time/h	0	4	8	12	24
PCD/%	$1.25 \pm 0.13^{(1)}$	$15.93 \pm 1.26^{(2)}$	$17.10 \pm 0.46^{(2)}$	$18.66 \pm 0.60^{(2)}$	$29.73 \pm 1.22^{(2)}$

(1) $\bar{x} \pm s$ ($n=6$) (2) $p < 0.01$ vs 0 h

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Tab 2. Changes in PCD of thymocytes 24 hours after different doses of in vitro X-irradiation

Dose	Apoptosis/%	P(vs control)
Control	20.15±1.50 ⁽¹⁾	
50 mGy	22.07±1.75	>0.05
75 mGy	20.36±1.23	>0.05
100 mGy	22.01±1.31	<0.05
200 mGy	25.27±1.21	<0.001
0.5 Gy	28.28±1.99	<0.0001
1.0 Gy	31.37±4.27	<0.001
2.0 Gy	31.40±3.50	<0.0001
4.0 Gy	33.36±5.01	<0.001
6.0 Gy	78.34±0.99	<0.001

(1) $\bar{x} \pm s, n = 6$

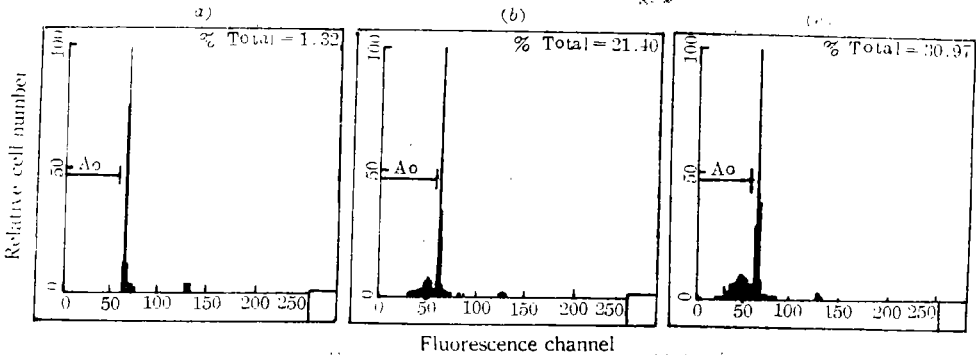


Fig 1. An example of histogram of cell cycle analysis of mouse thymocytes. PCD is shown in the Ao region
(a) After culture (b) Culture 12 h (c) Culture 24 h

大剂量辐射增加胸腺等器官的 PCD 已被公认。但小剂量辐射对 PCD 的影响尚待澄清。曾报道，0.05 Gy 体外照射放射敏感性极高的胸腺瘤细胞，可引起 PCD 增加^[6]。而本文用 FCM 所得实验结果表明，0.1 Gy 以上的剂量才会使小鼠正常胸腺细胞 PCD 百分率显著升高。同时还发现在全身照射的条件下，引起小鼠胸腺细胞 PCD 增多需要更高的剂量（将另文报道）。曾有人提出，小剂量辐射对免疫功能的刺激效应，是由于胸腺原始细胞 PCD 增多，继发地引起 T 细胞前体增殖所致^[6]。但现在已证实，0.1 Gy 以内的剂量全身照射可引起明显的免疫增强^[7]。因此，要阐明胸腺细胞 PCD 在小剂量辐射兴奋效应发生机制中的意义，还需要对比整体和离体照射条件下 PCD 发生的特点。

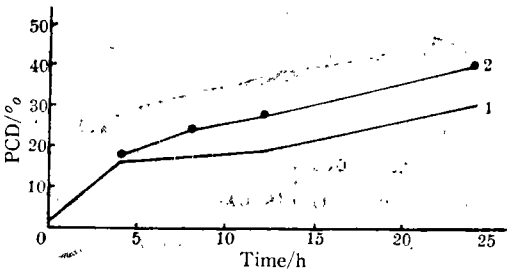


Fig 2. Changes of PCD in control and X-irradiated thymocytes in vitro
(1) Control (2) 2.0 Gy

参 考 文 献

- 1 Wyllie A et al. *Int. Rev. Cytol.*, 1980, 68: 251
- 2 Cohen J J, *Adv. Immunol.*, 1994, 50: 55
- 3 Garvy B A et al. *Immunology*, 1993, 79: 270
- 4 刘伟宏, 刘树铮. *生物化学与生物物理进展*, 1990, 17: 71
- 5 Ohyama H et al. in "Low dose irradiation and biological defense mechanisms" (Eds. T. Sugahara et al), Elsevier Science Publishers, 1992, pp: 361~364
- 6 Kondo S. *Int. J. Radiat. Biol.*, 1988, 53: 95
- 7 Liu S Z et al. *Health phys.*, 1987, 52: 579

RADIATION-INDUCED APOPTOSIS IN THYMOCYTES AS DETERMINED BY FLOW CYTOMETRY

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ABSTRACT Programmed cell death(PCD), or apoptosis, is a conceptually different way of cell death from necrosis. PCD plays an important role in immunologic regulation. PCD in thymocytes was analyzed by flow cytometry following in vitro X-irradiation. It was found that culturing of thymocytes could induce PCD which showed a time dependent increase. Four hours after culturing, 16% of thymocytes was found in the Ao region (PCD is shown in the Ao region of the histogram of flow cytometry). PCD in thymocytes showed a time dependent increase after 2.0 Gy X-irradiation, being significantly higher than that in the control at the same culturing time. 24 hours after X-irradiation in vitro, it was found that with doses below 100 mGy PCD was not significantly different from the control at the same culturing time, but when the doses were above 100 mGy, PCD showed a dose dependent increase, being significantly higher than that of the control at the same culturing time. These results are important in the understanding of the biological effects of low dose radiation.

KEYWORDS Programmed cell death (PCD), Flow cytometry (FCM), Thymocytes, X-rays