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【基础研究】

# X线放射后 *BALB/c* 小鼠 mCD99L2-A20 细胞成瘤率及外周血 T 淋巴细胞亚群变化

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**摘要: 目的** 探讨X线放射对 *BALB/c* 小鼠外周血 T 淋巴细胞亚群及 mCD99L2-A20 细胞成瘤率的影响。**方法** 40只 *BALB/c* 小鼠随机分为放射组和对照组,每组20只。放射组小鼠接受X线放射处理,对照组小鼠不接受X线放射处理。2组小鼠每只皮下注射接种 mCD99L2-A20 细胞悬液 100 μL($1\times10^7$ 个细胞),观察2组小鼠的成瘤情况;分别选择对照组、放射组接种 mCD99L2-A20 后成瘤及未成瘤小鼠各5只,于接种后20 d抽取小鼠外周静脉血,应用流式细胞仪检测 T 淋巴细胞亚群水平。**结果** 放射组小鼠成瘤13只(放射后成瘤组),未成瘤7只(放射后未成瘤组),成瘤率为65.0%;对照组小鼠成瘤1只,未成瘤19只,成瘤率为5.0%;放射组小鼠成瘤率显著高于对照组($\chi^2=15.824$,$P=0.000$)。对照组、放射后未成瘤组和放射后成瘤组小鼠外周血 $CD3^+$、$CD4^+$ 水平比较差异均无统计学意义($F=2.857$、$2.755$,$P=0.116$、$0.104$),但3组小鼠外周血 $CD8^+$ 水平比较差异有统计学意义($F=65.087$,$P=0.000$)。放射后未成瘤组和放射后成瘤组小鼠外周血 $CD8^+$ 水平显著低于对照组($t=6.166$、$12.982$,$P=0.000$),放射后成瘤组小鼠外周血 $CD8^+$ 水平显著低于放射后未成瘤组($t=4.597$,$P=0.002$)。**结论** X线放射后 *BALB/c* 小鼠接种 mCD99L2-A20 细胞成瘤率提高,其机制可能与机体免疫功能抑制及细胞毒性 T 细胞减少有关。

**关键词:** *BALB/c* 小鼠;X线放射;T淋巴细胞亚群;成瘤率
**中图分类号:** R392.12 **文献标志码:** A **文章编号:** 1004-7239(2016)04-0254-04

## Rate of tumor formation of mCD99L2-A20 cells and changes of peripheral blood T lymphocyte subsets of *BALB/c* mice after X-ray radiation

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**Abstract: Objective** To investigate the effect of X-ray radiation on the rate of tumor formation of mCD99L2-A20 cells and changes of peripheral blood T lymphocyte subsets in *BALB/c* mice. **Methods** Forty *BALB/c* mice were randomly divided into radiation group and control group, with twenty rats in each group. The mice in radiation group received X-ray radiation, but the mice in control group did not accept X-ray radiation. mCD99L2-A20 cell suspension($1\times10^7$ cells) were inoculated subcutaneously to the *BALB/c* mice in the two group, then the rate of tumor formation of mice was observed in the two groups. Five normal mice(control group), five mice with tumor formation and five mice without tumor formation(radiation group) were selected. The peripheral blood T lymphocyte subsets were detected by flow cytometer twenty days after inoculation. **Results** There were thirteen mice with tumor formation(tumor formation group) and seven mice without tumor formation(no tumor formation group) in radiation group, the tumor formation rate was 65.0%. There were one mice with tumor formation and nineteen mice without tumor formation in control group, the tumor formation rate was 5.0%. The tumor formation rate of mice in radiation group was significantly higher than that in control group($\chi^2=15.824$, $P=0.000$). There was no significant difference in the levels of $CD3^+$, $CD4^+$ in peripheral blood among control group, no tumor formation group and tumor formation group($F=2.857$, $2.755$; $P=0.116$, $0.104$). There was significant difference in peripheral blood $CD8^+$ level of mice among the three groups($F=65.087$, $P=0.000$). The $CD8^+$ level in peripheral blood of mice in tumor formation group and no tumor formation

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group was significantly lower than that in control group($t = 6.166, 12.982$; $P = 0.000$). The $CD8^+$ level in peripheral blood of mice in tumor formation group was significantly lower than that in no tumor formation group($t = 4.597$, $P = 0.002$). **Conclusions** The tumor formation rate of mCD99L2-A20 cells increase in *BALB/c* mice after X-ray radiation. The mechanism may be related to the inhibition of immune function and the decrease of cytotoxic T cells. Following radiation immunosuppression of T cells might have been related to suppressed immune function and reduced cytotoxic T cells in the organism.

**Key words**: *BALB/c* mice; X-ray radiation; T lymphocyte subsets; tumor formation rate

恶性淋巴瘤是原发于淋巴系统的一类疾病,A20 细胞是一种鼠 B 淋巴瘤细胞株,有报道证明下调 CD99 基因可诱导 B 淋巴瘤细胞转化为 Hodgkin/Reed-sternberg(HRS)细胞[1]。作者所在课题组前期将慢病毒短发夹 RNA(short hairpin RNA,shRNA)质粒转染内源性 mCD99L2(mouse CD99 antigen-like 2)基因表达阳性的鼠 B 淋巴瘤细胞株 A20,筛选出低表达 mCD99L2 基因的 mCD99L2-A20 克隆株,在将其皮下接种于同源性 *BALB/c* 小鼠时发现该细胞成瘤率非常低[2-3]。为此,本研究在将 mCD99L2-A20 细胞接种前对 *BALB/c* 小鼠进行全身 X 线放射,改变小鼠 T 淋巴细胞的免疫功能,从而提高了 *BALB/c* 小鼠的成瘤率。

## 1　材料与方法

**1.1　细胞来源及培养**　mCD99L2-A20 细胞由南方医科大学病理学系构建并鉴定[2-4]。mCD99L2-A20 细胞应用含体积分数 10% 胎牛血清的 RPMI-1640 培养基,于 37 ℃、含体积分数 5% $CO_2$ 培养箱内培养。收集生长状态良好的细胞悬液,无血清培养基清洗 3 次,再用无血清培养基重悬细胞,显微镜下计数,制备好的细胞悬液放置于冰盒内,于 30 min 内尽快完成接种。

**1.2　实验动物及分组**　无特定病原体级 *BALB/c* 小鼠 40 只,4 ~ 5 周龄,雌雄不限,体质量(18.0 ± 3.0)g;购自南方医科大学实验动物中心,合格许可证号:SCXK(粤)2006-0015。小鼠饲养于恒温(20 ~ 26 ℃)、恒湿(50% ~ 56%)、无特定病原体的空气洁净层流架内,按清洁级动物饲养标准进行喂养。40 只 *BALB/c* 小鼠随机分为放射组和对照组,每组 20 只。

**1.3　*BALB/c* 小鼠放射处理**　放射组小鼠接受直线加速器(瑞典 Elekta 公司)X 线放射,采用源-轴间距照射技术,X 线能量为 8 mv,照射野面积为 14.70 cm × 30.06 cm,肿瘤吸收剂量为 2 Gy,放射处方剂量 768.18 mu,照射深度 1.5 cm,剂量率:250 mu · $min^{-1}$,照射时间为 4.5 min。对照组小鼠不进行 X 线放射。

**1.4　mCD99L2-A20 细胞接种及成瘤情况观察**　于放射后 48 h 内,每只小鼠皮下(前肢腋下)注射接种 mCD99L2-A20 细胞悬液 100 μL($1 \times 10^7$ 个细胞),观察 2 组 *BALB/c* 小鼠的成瘤情况。

**1.5　小鼠外周血 T 淋巴细胞亚群检测**　接种细胞 20 d 后,分别选择对照组、放射组接种 mCD99L2-A20 后成瘤及未成瘤小鼠各 5 只,抽取小鼠外周静脉血(均自眼球取血)3 mL,置于肝素抗凝管中,每份样品重复 3 次,每管分别加入全血 100 μL,再按组分别加入荧光标记的抗小鼠单克隆抗体(美国 Invitrogen 公司)CD3-PE(5 μL)、CD4-FITC(2 μL)、CD8-PE-cy5(5 μL),室温(18 ~ 25 ℃)中孵育 15 min,应用流式细胞仪(美国 Backman BD 公司)检测 T 淋巴细胞亚群。

**1.6　统计学处理**　应用 SPSS 20.0 软件进行统计分析,计量资料以均数 ± 标准差($\bar{x} \pm s$)表示,组间 T 淋巴细胞亚群比较采用单因素方差分析(one-way ANOVA)和 LSD-$t$ 检验多重比较法,成瘤率比较采用 $\chi^2$ 检验,$P < 0.05$ 为差异有统计学意义。

## 2　结果

**2.1　2 组小鼠成瘤率比较**　放射组小鼠成瘤 13 只(放射后成瘤组),未成瘤 7 只(放射后未成瘤组),成瘤率为 65.0%;对照组小鼠成瘤 1 只,未成瘤 19 只,成瘤率为 5.0%;放射组小鼠成瘤率显著高于对照组,差异有统计学意义($\chi^2 = 15.824$, $P = 0.000$)。

**2.2　各组小鼠外周血 T 淋巴细胞亚群比较**　结果见表 1 和图 1。对照组、放射后未成瘤组和放射后成瘤组小鼠外周血 $CD3^+$、$CD4^+$ 水平比较差异均无统计学意义($F = 2.857$、$2.755$, $P = 0.116$、$0.104$),但 3 组小鼠外周血 $CD8^+$ 水平比较差异有统计学意义($F = 65.087$, $P = 0.000$)。放射后未成瘤组和放射后成瘤组小鼠外周血 $CD8^+$ 水平显著低于对照组,差异均有统计学意义($t = 6.166$、$12.982$, $P = 0.000$),放射后成瘤组小鼠外周血 $CD8^+$ 水平显著低于放射后未成瘤组,差异有统计学意义($t = 4.597$, $P = 0.002$)。

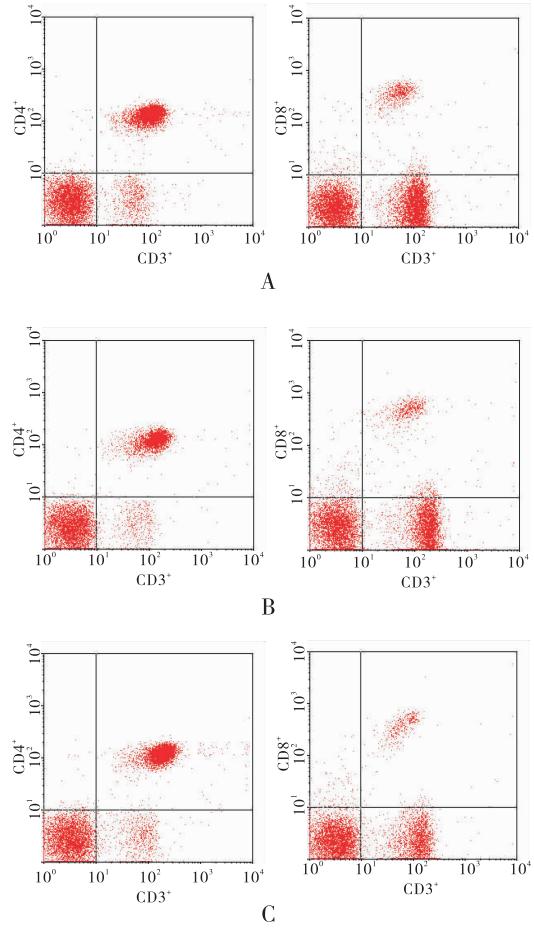
**表 1 3 组 *BALB/c* 小鼠外周血 T 淋巴细胞亚群比较**

**Tab. 1 Comparison of the levels of T lymphocyte subsets in peripheral blood of *BALB/c* mice among the three groups**

($\bar{x} \pm s$)

| 组别 | $n$ | $CD3^+$/% | $CD4^+$/% | $CD8^+$/% |
|---|---|---|---|---|
| 对照组 | 5 | 58.80 ± 7.07 | 50.70 ± 3.24 | 10.36 ± 0.67 |
| 放射后未成瘤组 | 5 | 50.64 ± 4.61 | 46.00 ± 4.45 | 7.44 ± 0.79[a] |
| 放射后成瘤组 | 5 | 57.86 ± 6.65 | 51.82 ± 4.65 | 5.58 ± 0.53[ab] |
| $F$ | | 2.587 | 2.755 | 65.087 |
| $P$ | | 0.116 | 0.104 | 0.000 |

注：与对照组比较[a] $P = 0.000$；与放射后未成瘤组比较[b] $P = 0.000$。




A：对照组；B：放射后未成瘤组；C：放射后成瘤组。

**图 1 流式细胞仪检测各组 *BALB/c* 小鼠外周血 T 淋巴细胞亚群**

**Fig. 1 Flow cytometry detection of T lymphocyte subsets in peripheral blood of *BALB/c* mice in each group**

## 3 讨论

研究显示，将慢病毒 shRNA 质粒转染内源性 mCD99L2 基因表达阳性的鼠 B 淋巴瘤细胞株 A20，筛选出低表达 mCD99L2 基因的 mCD99L2-A20 克隆株[2-3]，但在皮下接种于同源性 *BALB/c* 小鼠时发现该细胞成瘤率非常低。因此，本研究在将 mCD99L2-A20 细胞接种前对 *BALB/c* 小鼠进行全身 X 线放射，抑制并破坏体内的免疫系统，以此来诱导小鼠成瘤。恶性淋巴瘤是原发于淋巴系统的一类疾病，来源于 B 淋巴细胞、T 淋巴细胞或自然杀伤细胞的非正常克隆性增殖，近年来随着获得性免疫缺陷综合征，器官移植，肿瘤放射治疗、化学治疗免疫抑制的应用[5]，发病率急剧升高。H/RS 细胞来源 B 淋巴细胞，其发生与 CD99 基因表达缺失有关，本课题组前期成功诱导低表达 mCD99L2 基因的 mCD99L2-A20 克隆株，其类似人 H/RS 细胞且具有 H/RS 细胞表型。本研究结果显示，放射组和对照组小鼠成瘤率分别为 65.0% 和 5.0%，因 *BALB/c* 小鼠具有完整的 T 细胞和 B 细胞免疫功能，在正常状态难以成瘤，所以放射组小鼠成瘤率显著高于对照组；对照组、放射后未成瘤组和放射后成瘤组小鼠外周血 $CD3^+$、$CD4^+$ 水平比较差异均无统计学意义，但 3 组小鼠外周血 $CD8^+$ 水平比较差异有统计学意义；放射后未成瘤组和放射后成瘤组小鼠外周血 $CD8^+$ 水平显著低于对照组，放射后成瘤组小鼠外周血 $CD8^+$ 水平显著低于放射后未成瘤组。$CD8^+$ 细胞是以细胞毒性 T 淋巴细胞（cytotoxic lymphocyte，CTL）为主，其水平降低可能导致：（1）对瘤细胞的直接杀伤能力大大降低，有利于肿瘤形成；（2）CTL 减少，辅助性 T 淋巴细胞（T helper lymphocyte，Th）相对增多，有利于介导瘤细胞形成免疫耐受，从而逃避免疫监视，促进瘤体的形成[4,6]；（3）$CD8^+$ 分泌的大量炎性因子（如 γ 干扰素、趋化因子等）减少[7]，γ 干扰素不仅是单核巨噬细胞强劲的活化剂，还可同时诱导多种细胞表达主要组织相容性复合体Ⅰ和Ⅱ分子，同时也是促进 T 细胞分化和 $CD8^+$ 细胞毒性 T 细胞成熟的重要因子，γ 干扰素减少有利于肿瘤形成[8]。此外，瘤细胞可以通过广泛的细胞因子、趋化因子网络吸引大量的 T 淋巴细胞浸润[9-12]，这些因子可以限制向 $CD8^+$ 细胞毒性 T 细胞递呈抗原，逃避 CTL 杀伤[13]，同时可以促进 Th 细胞向 Th2 亚群分化，间接抑制免疫功能，介导免疫耐受[14-16]。本实验结果提示，将慢病毒 ShRNA 质粒转染内源性 mCD99L2 基因表达阳性的鼠 B 淋巴瘤细胞株 A20，筛选出低表达 mCD99L2 基因的 mCD99L2-A20 克隆株，在皮下接种难以成瘤时，接种瘤细胞前对小鼠进行全身 X 线放射，改变小鼠体内的 T 淋巴细胞亚群，尤其降低 $CD8^+$ 水平，可明显提高成瘤率，T 淋巴细胞在小鼠成瘤过程中发挥了重要作用，但其具体作用机制仍需进一步研究证实。

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