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(E10). Brain specimens from rat offsprings were collected on the 18th day of gestation(E18)and postnatal day 0 (P0). The expression of neural cell adhesion molecule NCAM and L1 in cerebral cortex was assayed with immunocytochemical staining to study effects of beta radiation from HTO on neuronal migration in offsprings. Morris water maze was used to detect the changes of brain function during P38 ~ P42. The proton magnetic resonance spectroscopy (^{1}H-MRS) was used to measue the ratio of Cho/Cr (choline/creatine) and NAA/Cr (N-acetyl aspartate/creatine) in cerebral cortex on P90 to analyse the distribution of neural cells. ***Results*** Compared with control, the expression of neural cell adhesion molecule NCAM and L1 in brains of experimental offsprings(E18 and P0)was significantly reduced ($P < 0.05$). The escape latency of experimental offsprings was significantly prolonged ($P < 0.05$). The swimming time in the quadrant of platform was obviously shortened in the probe trial ($P < 0.05$). The ratio of NAA/Cr in experimental group was significantly decreased ($P < 0.05$) and there was no difference between Cho/Cr ratios in two groups ($P > 0.05$). ***Conclusion*** The results suggest that HTO irradiation in utero could induce brain disfunction and disturb the distribution of neuronal cells in offspring. The mechanisms may be related to the downregulation of neural cell adhesion molecule NCAM and L1.

Key words tritiated water; ionizing radiation; neuronal migration; Morris water maze; proton magnetic resonance spectroscopy

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◇**学位论文摘要**◇

乳铁蛋白抗菌-乳源免疫调节融合肽的表达纯化及其基因对SKOV3细胞的影响

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摘要 目的 构建并鉴定牛乳铁蛋白抗菌-乳源免疫调节融合肽(简称LIFP)基因的重组载体,在大肠杆菌BL21中诱导表达和纯化,获得较高纯度的融合肽;研究LIFP基因体外对卵巢癌细胞SKOV3的影响,为基因治疗卵巢癌提供基础。**方法** 以GENBANK报道的牛乳铁蛋白抗菌肽(LFCINB)与乳源免疫调节肽(PGPIPN)的序列为参照,以连接臂GGGGS连接两种肽,合成融合肽LIFP基因,并在其5'端加入凝血酶FXA识别的酶切位点。将LIFP基因构建到表达载体PGEX-KG中,PCR筛选阳性克隆,测序鉴定后,采用最优表达条件诱导重组载体在大肠杆菌BL21中表达,超声裂菌后,SDS-PAGE和Western-blot鉴定融合蛋白(LIFP-GST),通过AKTA层析系统纯化融合蛋白,FXA酶切后,高效液相色谱(HPLC)、质谱(MS)鉴定LIFP的表达;将LIFP基因构建到PLJM1载体[含CMV启动子和绿色荧光蛋白(GFP)中,获得PLJM1-LIFP慢病毒表达载体,经PCR检测及测序鉴定后,与慢病毒包装质粒通过LIPOFEETAMINE 2000共转染至包装细胞293T,包装产生病毒液,并测定其滴度;体外培养人卵巢癌细胞SKOV3,重组慢病毒感染细胞后,MTT法观察融合肽对SKOV3细胞的生长抑制作用,HOECHST33258染色观察细胞核形态的变化。**结果** PCR和测序证实成功构建了PGEX-KG-LIFP重组载体;WESTERN-BLOT结果显示成功诱导LIFP的高表达;HPLC显示获得较高纯度LIFP;MS显示氨基酸序列与目的肽一致;成功构建PLJM1-LIFP慢病毒表达载体,三质粒共转染293T细胞后,荧光显微镜下可见大量绿色荧光。包装慢病毒产生病毒悬液的滴度为1.2×10^8 TU/ml;重组病毒感染SKOV3细胞后,MTT结果显示融合肽对体外培养的SKOV3细胞生长具有抑制作用($P < 0.05$),荧光显微镜观察到凋亡细胞典型的形态学特征。**结论** 成功诱导表达纯化融合肽,并初步证实对体外SKOV3有生长抑制和诱导细胞凋亡作用。

关键词 融合肽;表达;纯化;慢病毒载体;细胞凋亡

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