13

MONITORING EMBRYONIC STEM CELL TRANSPLANATATION INTO RAT CORPUS CAVERNOSUM BY USING OPTICAL IMAGING SYSTEM

Departments of Nuclear Medicine[1] and Urology[2], Chonnam National University Medical School, Department of Physiology[3], Chonnam National University College of Veterinary Medicine

Jung-Joon Min[1] *, Sungmin Moon[1], Uyenchi N. Le[1], Kwangsung Park[2], Ho Jae Han[3], Hyun Suk Lee[2], Ho Cheon Song[1], Hee-Seung Bom[1]

Purpose: The conventional method for the analysis of stem cell transplantation depends on postmortem histology. Here, we have sought to demonstrate the feasibility of a longitudinal monitoring of transplanted cell survival in living animals, by employing optical imaging techniques. **Methods:** Mouse embryonic stem cells (ESC) were obtained from American Type Culture Collection (ES-E14TG2a). Mouse ES cells were cultured in the DMEM (Gibco-BRL, Gaithersburg, MD) supplemented with 3.7 g/L sodium bicarbonate, 1% penicillin and streptomycin, 1.7 mM L-glutamine, 0.1mM β-mercaptoethanol, 5 ng/mL mouse leukemia inhibitory factor (LIF), and 15% fetal bovine serum (FBS) with or without a feeder layer and cultured for five days in standard medium plus LIF. ESCs were then transfected (MOI=100) overnight with Ad-CMV-Fluc. Our experimental Sprague-Dawley rats (n=7) were given with different numbers of ESCs (10^5, 10^6, 5x10^6) expressing Fluc into corpus cavernosum. Sham-operated rats were controls (n=6). Cell survival was assessed histologically and/or by optical bioluminescence imaging which was conducted using a cooled charged-coupled device camera (Xenogen), beginning on the day after the transplantation. **Results:** In cell cultures, firefly luciferase activity correlated linearly with cell numbers from 10^5 to 5x10^6 (r2=0.95). In living animal imaging, imaging signal activity correlated linearly with cell numbers injected from 10^5 to 5x10^6 at each time point (r2=0.62 ~0.98). In all three groups of rats, imaging signal was detected in rat genital area from the 2nd day to the 47th day after cellular injection. **Conclusion:** Adenovirus mediated transient expression of firefly luciferase reporter gene in ESCs was feasible to monitor cell survival over a month after transplantation. The locations, magnitude, and survival duration of the ESCs were noninvasively monitored with a bioluminescence optical imaging system.

14

Alpha-fetoprotein Specific Enhancer에 의해 발현되는 유전자 Construct 제작

경북대학교병원 핵의학과[1], 경북대학교병원 내분비내과[2]

류민정[1] *, 안손주[1], 윤선미[1], 최승희[1], 안병철[1], 이인규[2], 이재태[1], 이규보[1]

목적: 특정세포에서만 발현이 되는 특이 프로모터에 치료용 유전자를 접목시켜 악성종양을 치료하려는 유전자 치료법이 지속적으로 시도되고 있다. 본 연구는 간암세포에서만 특이적으로 파괴하는 유전자치료용 adenovirus를 제작하기 위한 기본 연구로써, 간암세포에서만 특이 발현되는 Alpha-fetoprotein (AFP) promoter의 조절을 받는 치료용 유전자를 제작기술 개발을 위해 시행되었다. **방법:** AFP promoter region의 -4.1~-3.3kb site에 존재하는 enhancer를 Genomic DNA에서 PCR (Forward: CGAGCTCATTTTCATGATATAG, Reverse : CTAGCTAGCTAGATAAGGAGAC)을 통해 selection하였다. pGL3- promoter 벡터에 클로닝하였고 유전자 construct를 확보하였다. 세포 특이발현을 평가하기 위하여 유전자 construct를 간암세포주인 HepG2, Hep3B와 비간암세포주인 C2C12 (근육배아세포)에서 Luciferin을 이용한 인광측정 방법을 이용하였고 triplicate로 luciferase activity를 측정하였다. **결과:** AFP enhancer을 luciferase와 함께 pGL3-promoter 벡터에 도입하였다. 이 벡터을 간암세포주 및 비간암세포주에 동시에 transfection 시킨 후 배양하여 luciferase acitivity를 측정해본 결과, 비간암세포주에 비해 간암세포주에서 luciferase activity가 3배 정도 높게 나타났다. **결론:** Alpha-fetoprotein (AFP) enhancer의 조절을 받는 lucifease 유전자를 제작하였다. 현재 사용된 luciferase gene 을 NIS 유전자로 대치하면 간암세포에서만 특이적으로 파괴하는 유전자치료용 adenovirus를 제작할 수 있을 것으로 판단된다.