Non-invasive monitoring of carcinogenesis in N-nitrosodiethylamine induced liver cancer

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1. Introduction

Molecular imaging based on reporter gene expression allows tissue-specific events or processes to be measured using the bioluminescence imaging (BLI) reporter gene expression vector controlled by specific enhancer/promoters [1, 2]. Alpha-fetoprotein (AFP), which is a tumor marker, is a serum glycoprotein that is expressed normally by fetal liver and yolk-sac cells, as well as in trace amounts in the fetal gastrointestinal tract. The serum concentration of AFP decreases rapidly after birth and its expression is repressed in adults. Approximately 80% of HCC patients show an increase in the AFP level [3]. Therefore, AFP has been used for many years as a diagnostic and prognostic serum marker for HCC [4] and transgenic system for AFP was proposed as a valuable tool for elucidation of mechanism of transcriptional regulation during liver development and hepatocarcinogenesis [5]. In this study, firefly luciferase (fLuc) expressing transgenic mice controlled by the AFP enhancer/ promoter (enh/promoter) were produced to screen for the development of AFP-producing liver cancer. These models are expected to be useful for monitoring agents or drugs that modulate the AFP level as well as for measuring the specific signaling events important for liver cancer development.

2. Methods and Results

2.1 In vitro study

The AFP-producing human HCC cell line, HuH-7 (JCRB 0403) and the AFP-nonproducing human embryonic kidney cell line, 293A (ATCC CRL-1573), were chosen for this comparative study of reporter gene expression according to the levels of AFP expression.

The pGL-Basic vector (Promega, WI, USA) was used to make a construct containing the 2.1 Kb of the alpha-fetoprotein enh/promoter. The AFP enh/promoter gene was amplified from the plasmid, pDRIVE-AFP-hAFP (Invivogen, CA, USA) using a polymerase chain reaction and then inserted into the Mlu I and Xho Ⅰ sites of pGL-Basic.

After 24 hours transfection, the cells were washed with phosphate buffered saline (PBS) and harvested using a lysis solution (Tropix, MA, USA). The cell lysates with luciferase substrate A (ATP) and B (D-luciferin) were placed on an opaque 96 well plate and measured using a Luminometer (Spectramax, Molecular devices, CA, USA). The analysis program utilized was Softmax pro5.2 (Molecular devices, CA, USA). After transfection of pAFP-fLuc, the fLuc activity in the AFP-producing HCC cells was higher than that in the AFP-nonproducing cells.

2.2 Generation of transgenic mice

Transgenic mice were generated at Macrogen (Seoul, Korea). The transgenic mice were generated by the microinjection of a linear transgene containing AFP enh/promoter-fLuc sequence, in which pAFP-fLuc was digested with Mlu Ⅰ and Sal Ⅰ into super-ovulated fertilized eggs of C57BL/6 mice. The presence of the transgene in the founders was confirmed by PCR using the genomic DNA extracted from the tail. Eleven positive founders out of 110 founders were obtained from genomic PCR typing.

2.3 In vivo bioluminescence image (BLI)

All BLIs were acquired using an IVIS imaging system series 200 (Xenogen, CA, USA) to detect the bioluminescence signals in the transgenic mice. BLIs of neonatal progenies of positive founders were acquired to confirm the fLuc activity. Each neonatal progeny was injected intraperitoneally with 3 mg of D-luciferin (Molecular Imaging Products Company, OR, USA) in PBS and the BLIs were acquired immediately over a 1 min period. To measure the intensity of emitted light, a region of interest (ROI) were drawn over the emitted region and the total photon efflux was determined. The bioluminescent signals are expressed in units of photons per cm2 per second per steradian (p/cm2/s/sr). Positive progenies of 4 lines showed a strong bioluminescence signal in their liver region in the fetus as well as at 1 day after birth (Fig 1).

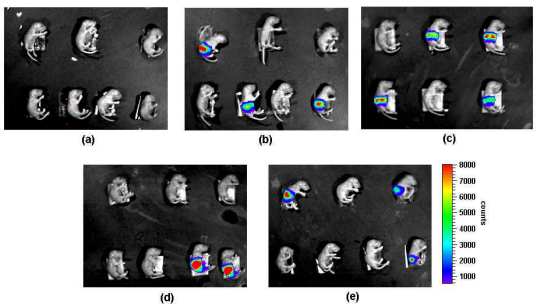

Fig. 1. BLIs of their progenies of wild-type(a) and 4 positive founders (founder numbers 39, 59, 67 and 78, (b),(c),(d) and

(e)). Each positive founder was mated with wild-type female or male partner. One day after birth, their neonatal progenies were anesthetized and intraperitonial injected with D-luciferin.

2.4 Measurement of AFP expression level in neonatal liver

After BLIs of neonatal progenies from 1 to 10 days after birth were acquired, mice were sacrificed and RT-PCR was performed using total RNA from neonatal liver to detect AFP level. AFP expression level was detected from band intensity of RT-PCR products on agarose gel (Gel-pro Analyzer 3.1, Media Cybernetics, MD, USA) and presented as a relative band intensity of AFP PCR product to internal control. The BLI signals of the neonatal progeny mice decreased according to the time passed after birth. By quantifying the light intensity, a rapid decrease in fLuc activities was observed in the liver region (Fig 2, b).

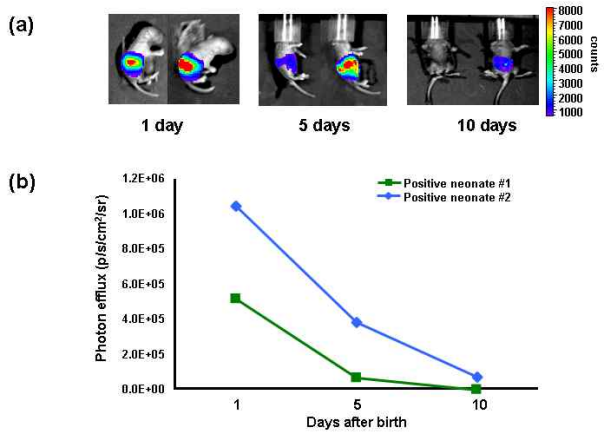

Fig. 2. BLIs of the neonatal progenies according to passed time after birth. BLIs of neonatal progenies from positive founder 39 were obtained 1 day, 5 days and 10 days after birth. At 10 days after birth, BLI of littermate rapidly was declined. (a); Longitudinal BLI of neonate after birth (b); The quantified BLI signal on the emitted region. The fLuc activities during passed time after birth were decreased by region of interest (ROI) measurement

2.5 Chemical induced liver cancer model

We aimed to be evaluating liver cancer in mice model induced by N-nitrosodiethylamine (DEN) as a chemical carcinogen. The carcinogen-induced mice model was developed by intraperitoneal injection of DEN in transgenic mice. These transgenic mice were injected once with 20 mg/kg body weight of DEN at 3 weeks old mice. Liver cancer was developed at 36 weeks of age determined with 3T MRI and histologic evaluation. BLI of liver cancer induced transgenic mouse showed high BLI activity in liver region (Fig 3).

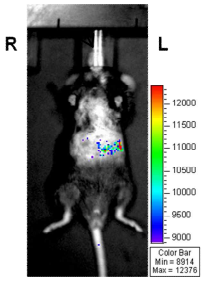

Fig. 3. BLI of liver cancer induced transgenic mouse by DEN.

3. Conclusions

In conclusion, a transgenic mouse model for imaging AFP-producing cells or organs by BLI was developed using an AFP enh/promoter and fLuc. The images correlated well with the bioluminescence signals in the liver and AFP levels. This transgenic mouse model can be used to detect HCC without the need for sacrifice as well as for longitudinal monitoring of the hepatocarcinogenesis process in vivo. The transgenic mice will be also useful for screening modulating agents, such as chemicals, nucleotides, or natural extracts, for AFP expression level using in vivo BLI system.

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