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A stringent dual control system overseeing transcription and activity of the Cre recombinase for the liver-specific conditional gene knock-out mouse model

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Abstract

Liver cancer is one of the most threatening diseases in Chinese population. Just like in other tissues, tumor initiation and development in liver involve multiple steps of genetic and epigenetic alterations with several unknown details. However, unlike in other tissues, a tissue specific inducible Cre recombinase system that allows temporal and spatial deletion of a target DNA fragment is still not available for *in vivo* functional gene annotation in hepatocytes. In our pursuit to establish such a mouse model, we designed a dual inducible Cre transgene system and tested it in cultured cells. By combining a CCAAT/enhancer binding protein β (C/EBP β) promoter derived Tet-off expression system and the estrogen receptor (ER) mediated functional control, we show a desirable profile of both hepatocyte-specificity and regulability of the Cre expression in a series of critical assessments in the cell culture system, which provides confidence in continuation of our ongoing pursuit in mouse.

Keywords: Cre/loxP; Tet-off; hepatocyte-specific; doxycycline; 4-OHT; C/EBP β

Introduction

Hepatocellular carcinoma, accounting for over 70% liver malignancy, is one of the most devastating types of human cancer. It chiefly affects people in Far East Asia and Africa, the mainland of China in particular, where over 50% of the total incidence and mortality worldwide is reported (El-Serag and Rudolph, 2007). Same as other types of malignancy, hepatocellular carcinoma also involves multiple steps of genetic (Laurent-Puig and Zucman-Rossi, 2006) as well as epigenetic defects (Zhu, 2005; Feinberg, 2007; Zhu and Yao, 2007), presenting a formidable challenge to our capability of understanding the details of the key defects in the natural process and ultimately constraining the diseases. The genetically modified mouse models have been indispensable to our efforts to mechanically dissect and develop the robust therapeutics for this type of diseases (Frese and Tuveson, 2007).

The use of the Cre (causes recombination of the bacteriophage P1 genome) recombinase system that creates the *loxP* (locus of crossover(x) in P1) sequence (artificially integrated) specific chromosomal recombination (Rajewsky et al., 1996) for the cell type specific knock-out of the mouse equivalent of the human gene has revolutionized the practice of the *in vivo* function annotation of human genes during development as well as diseases (Branda and Dymecki, 2004; Glaser et al., 2005). Incorporation of the ligand (such as tetracycline (Gossen and Bujard, 1992)) mediated transcription switch on or off cassette allows a temporal control of the target gene expression, and therefore, effectively overcomes the hurdles associated with the embryonic lethality of several germ-line null mutations

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(Lewandoski, 2007). Using the promoter/enhancer of the genes that assume a cell lineage specific (selective) transcription to restrict the expression of transacting factor genes (in the Tet-on/Tet-off system) has facilitated the *in vivo* study (Utomo et al., 1999; Lewandoski, 2001; Schonig et al., 2002). The investigation in the mouse strains of a brain-specific and even the brain sub-region-specific expression of the *Cre* gene has already provided some deep insights in the functional role of several neuron-associated genes during development (Baumgartel et al., 2007; Britsch, 2007; Erdmann et al., 2007). Probably, owing to the inability to rationally assemble the transcription regulatory DNA elements, the leaky transcription of the *Cre* gene with detrimental toxic effect has prevented the creation of several cell type-specific conditional knock- out mouse strains (Schmidt-Supprian and Rajewsky, 2007). The noticeable absentee is the hepatocyte (liver) -specific Cre mouse model (personal communication with Dr. C. Deng and X. Wang in NIH, USA) (http://emice.nci.nih.gov/mouse_modls/mouse_publiations), which has held back the efforts to understand and control the key malignancy in liver: hepatocellular carcinoma.

It has also been possible to regulate the Cre recombinase activity by the approach developed by Feil et al. (1996). Fusion to a mutated ligand-binding domain (LBDs) of the estrogen receptor has made Cre protein complex with Hsp90, retain in cytoplasm, and functionally inactive. Cre recombinase activity is recovered when the synthetic estrogen analogues, 4-hydroxytamoxifen (4-OHT), but not the native estrogen breaks Cre-ERT from the Hsp90 sequestration and subsequently for transfer into nucleus. The stringency of the control has been further improved in the Cre-ERT2 fused recombinase where three amino acid changes (G400V/M543A/L540A) in LBDs have been introduced (Hirrlinger et al., 2006). Cre-ERT2 transgenic mouse lines specific to neurons (Erdmann et al., 2007), astrocytes (Hirrlinger et al., 2006), smooth muscle cells (Kuhbandner et al., 2000), and germ cells (Weber et al., 2003) have already been used for the better functional insights of several genes in human development and diseases.

In our pursuit to establish such a mouse model for the hepatocyte lineage, we designed a dual inducible Cre transgene system and tested it in cultured cells. It involved using a CCAAT/enhancer binding protein β (C/EBP β) promoter derived Tet-off expression system and the estrogen receptor (ER) mediated functional control of the recombinase activity of Cre-ERT2 protein (Feil et al., 1997) (Fig. 1). We showed a desirable profile of both hepatocyte-specificity and regulability of *Cre* expression in a series of

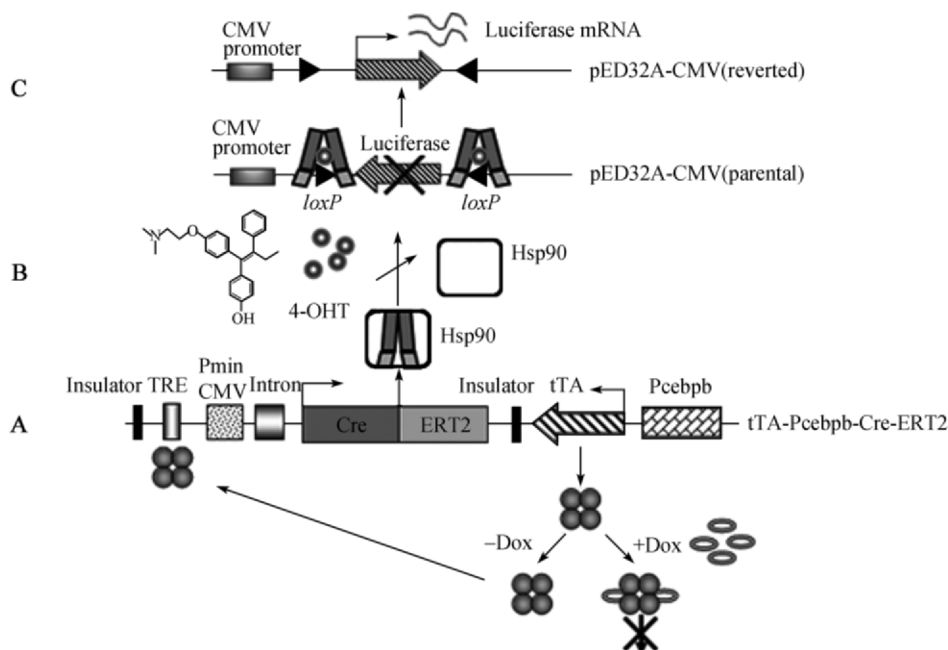

Fig. 1. The dual control systems for the hepatocyte-specific regulation of the Cre-ERT2 recombinase. A: a single unit form of Tet-off system where the *tTA* gene was driven by the promoter of the rat *C/EBP β* gene and was separated by a pair of insulators as indicated. Doxycycline (the more effective form of tetracycline, +Dox) prevented tTA from binding and activating the *Cre-ERT2* gene transcription under the control of both a minimal promoter (PminCMV) and 7 copies of the tetracycline responsive elements (TRE). Depletion of Doxycycline (–Dox) in medium would release tTA, so that transcription of the *Cre-ERT2* gene would be assumed. B: the Cre-ERT2 fusion protein was sequestered by heat-shock proteins (Hsp90) in cytoplasm, which would be released when 4-OHT destroyed the interaction between Hsp90 and the LBD domain of ER, the fused protein of which subsequently translocated into nucleus, to mediate the *loxP* based genetic recombination. C: the luciferase gene flanked with two *loxP* sequences at each ends (in a header to header arrangement) was in antisense direction to the CMV promoter in pED32A-CMV (parental, inactive form). The active Cre recombinase released by 4-OHT (B) would revert the luciferase gene to the same orientation as CMV promoter, so that the luciferase transcription would be assumed and luciferase activity in cytoplasm could be detected.

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