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No. 1

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Introduction:

β-actin (*ACTB*) is one of the genes expressed most abundantly and ubiquitously in a wide range of human nonmuscular tissues. Previous studies have demonstrated pronounced transcriptional activity in an approximately 500–600-bp-long human *ACTB* (*hACTB*) promoter region. This 500–600-bp-long promoter thus appears to be suitable for use within expression vectors to drive exogenous genes. The *hACTB* promoter may also have an advantage in directing long-term gene expression, because it is likely less prone to silencing by epigenetic changes including DNA methylation compared to viral promoters. However, systematic comparisons of its long-term activity with those of other promoters have been scarcely performed to our knowledge.

In this study, we sought to examine the transcriptional activity of a 550-bp-long *hACTB* promoter fragment in multiple human somatic cell lines over time for up to 60 days, and compare it with those of several commonly used promoters, with a particular focus on the CMV promoter.

Materials and Methods:

Plasmids in which EGFP expression was driven by one of the *hACTB*, *hEF1α*, CAG, CMV, and HSV-TK promoters were constructed. Multiple human somatic cell lines were transiently or stably transfected with these plasmids and processed for the measurement of GFP signals by flow cytometry over time for up to 60 days. We also evaluated the long-term transcriptional activities of the *hACTB* and the CMV promoters using plasmids expressing the luciferase gene under the control of these promoters.

Results:

In the transient transfection of plasmids, the CMV promoter directed the highest GFP signals among all promoters analyzed. However, the CMV promoter directed faint GFP signals in stably transfected cells. In contrast, the *hACTB* promoter, which drove the second highest GFP expression in transiently transfected cells, maintained significantly greater GFP signals than the CMV promoter for up to 60 days. A sustained long-term transcriptional activity of the *hACTB* promoter was also observed in a luciferase-based assay.

Discussion:

There have been several studies comparing the transcriptional activity of the *hACTB* promoter with those of various cellular and viral promoters. However, the current study focused on the long-term activity of the *hACTB* promoter, and provided evidence that the use of the *hACTB* promoter ensures sustained, high-level expression of exogenous genes for a period up to 60 days, at least in some human cell lines.

Other cellular or synthetic promoters, such as the *hEF1α* and the CAG promoters, also demonstrated pronounced long-term transcriptional activities. However, the 550-bp-long *hACTB* promoter is about 1 kilobase shorter than these cellular/synthetic promoters commonly used in commercially available vectors, and thus likely to be useful for directing the expression of a large gene from a type of vector that has an upper size limit.

Conclusion:

This study demonstrated that a 550-bp-long *hACTB* promoter is an example of a cellular promoter with the ability to sustain long-term, high-level ectopic gene expression in human cell lines.