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Development of Gene-trap and Knockout Technology for Zebrafish

Seo-Ho Kim, Jeong-Soon Jeon, Tae-Hoo Kim and Ki-Young Lee

Faculty of Marine Life Science College of Ocean Science & Technology Kunsan National University San 68, Miryong-dong, Kunsan, Jeollabuk-do, 573-701, Korea

Introduction

Zebrafish, *Danio rerio* has increasingly become an important and a superb model organism for cellular and developmental studies, and functional genomics, as evidenced by the ongoing effort to sequence its whole genome. The significance of zebrafish functional genomics has been further enhanced by the finding of synteny conservation between zebrafish and human(Gates et al., 1999; Postlethwait and Talbot, 1997) and by the fact that zebrafish has produced a significant number of mutations that model human diseases(Dooley and Zon, 2000; Penberthy et al., 2002; Zon, 1999). Methods are available to perform large-scale mutagenesis screens, allowing identification of key regulatory genes in development. Nevertheless, to fully realize the potential of this organism, tools of reverse genetics are needed.

We have recently shown that fertile transgenic zebrafish can be produced by nuclear transfer (NT) using long-term cultured somatic cells. To utilize this technique for future characterization of novel genes and gene functions in zebrafish, we performed genetic manipulation on the cultured cells using the gene-trap strategy.

Meterials and Methods

We developed two retroviral gene-trap constructs. The first one (SA/GFP-TP) carries a GFP reporter gene containing a splicing acceptor and an internal neo gene. The second one (Neo-TP) contains a

promoter-less neo gene located in the LTR sequence of a viral vector. Pseudotyped retrovirus for each construct was generated to infect cultured zebrafish cells that had been used successfully in NT experiments.

Results and Discussion

In each case, neo-resistant clones were obtained after G418 drug selection. For SA/GFP-TP, approximately 0.2% cells of the neo-resistant population appeared positive for GFP expression, indicating generation of a GFP fusion protein (or proteins) by splicing. For Neo-TP, since the neo gene lacks a functional promoter, all of the neo-resistant cells should carry viral insertions immediately downstream of an active promoter. Molecular analysis indicates that gene-trap approaches can be used to identify expressed genes in cultured zebrafish cells(data not shown). As demonstrated in mouse, most insertions by promoter-less gene-traps will result in inactivation of the downstream genes. Therefore, we believe generation of live zebrafish carrying successful gene-trap events by NT should help to reveal functions of inactivated genes in vivo. We have also initiated experiments to perform gene knockout in the cultured embryonic fibroblast cells and plan to generate zebrafish carrying suck targeted mutation by animal cloning. If successful, zebrafish will provide all useful tools that have been used in mouse genetic studies.