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Research paper

Development of retroviral vectors for insertional mutagenesis in medaka haploid cells

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ABSTRACT

Insertional mutagenesis (IM) by retrovirus (RV) is a high-throughput approach for interrogating gene functions in model species. Haploid cell provides a unique system for genetic screening by IM and prosperous progress has been achieved in mammal cells. However, little was known in lower vertebrate cells. Here, we report development of retroviral vectors (rvSAchCVgfp, rvSAchCVpf and rvSAchSTpf) and establishment of IM library in medaka haploid cells. Each vector contains a modified gene trapping (GT) cassette, which could extend the mutated cell population including GT insertions not in-frame or in weakly expressed genes. Virus titration determined by flow cytometry showed that rvSAchSTpf possessed the highest supernatant virus titer (1.5×10^5 TU/ml) in medaka haploid cell, while rvSAchCVpf produced the lowest titer (2.8×10^4 TU/ml). However, quantification of proviral DNAs in transduced cells by droplet digital PCR (ddPCR) demonstrated that the "real titer" may be similar among the three vectors. Furthermore, an IM library was established by FACS of haploid cells transduced with rvSAchCVgfp at a MOI of 0.1. A single copy RV integration in the majority of cells was confirmed by ddPCR in the library. Notably, there was a significant decrease of haploid cell percentage after FACS, suggesting potential trapping for survival/growth essential genes. Our results demonstrated successful development of retroviral vectors for IM in medaka haploid cells, serving for haploid genetic screening of host factors for virus infection and genes underlying certain cellular processes in fish model.

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1. Introduction

A critical need to identify the set of genes underlying a cellular process has been raised since the completion of human and other model species genome projects. Large-scale screening using chemical mutagen (such as ENU, N-ethyl-N-nitrosourea) has successfully identified many genes involved in various developmental processes (Driever et al., 1996). However, the procedures are extremely laborious and time consuming. Insertional mutagenesis (IM) by gene trapping has been recently developed to elucidate gene functions by disrupting and recapitulating expression of genes in a target genome (Ivics et al., 2009). It enables a high-throughput screening of insertional mutants and isolated related genes with high efficiency and low cost.

Among approaches for IM, retroviral vectors can stably integrate into the target genome with high efficiency. Therefore, they have become the most commonly used vectors for IM (Yamaguchi et al., 2012). In fact, large-scale IM by pseudotyped retrovirus has been developed in zebrafish (Lin et al., 1994; Gaiano et al., 1996; Amsterdam et al., 1999). By this method, over 330 genes required for embryonic and early larvae development have been identified (Golling et al., 2002; Amsterdam et al., 2004; Amsterdam and Hopkins, 2006). However, it is difficult to screen novel genes involved in a cellular progress of interest under diploid circumstance, as IM in diploid cells often results in heterozygous mutants. In that time, researches have to use ES cell lines carrying Blm mutations, which cause frequent somatic crossing-over, to generate null mutants (Guo et al., 2004).

Recently, this hurdle has been overcome by haploid cells. Gene trapping by retroviral vector in nearly haploid human cancer cell line has generated insertions in >98% genes expressed in the cell (Carette et al., 2011a). Host factors essential for virus infection (Carette et al., 2009, 2011b; Papatheodorou et al., 2011; Rosmarin et al., 2012; Jae et al., 2013) and genes essential for various cellular processes (Carette et al., 2011a; Reiling et al., 2011; Birsoy et al., 2013; Lee et al., 2013; Timms et al., 2013) have been identified using the mutated cells. IM combined with haploid cell lines has become a powerful system, which will identify more and more novel genes involved in these kinds of cellular processes.

In spite of prosperous progress of haploid genetic screening in mammal cells, little was reported in lower vertebrate cells. Previously, we have reported generation of the world's first fish haploid cell line in medaka (Yi et al., 2009, 2010) which is sensitive to Singapore Grouper

Abbreviations: dpi or hpi, day(s) or hour(s) post infection; hpt, hours post transfection; MOI, multiplicity of infection; RV, retrovirus; IM, insertional mutagenesis; GT, gene trapping; ddPCR, droplet digital PCR; ST, SV40 early gene enhancer plus thymidine kinase promoter; TAG, transcriptional active gene.

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Iridovirus (SGIV), a highly infectious fish virus in aquaculture (Yuan et al., 2013). As a fish model, the cell line therefore can be applied to generate IM library and screen host factors for SGIV infection. The progress on identifying host factors and mechanisms underlying virus–host interactions will guide for establishing new anti-virus strategies for aquaculture (Zhang and Gui, 2015). As a first step, here we aim to develop retroviral vectors and procedures to establish IM library using medaka haploid cells, serving for haploid genetic screening of host factor for virus infection and cellular processes in the model.

2. Materials and methods

2.1. Retroviral plasmid construction

All the retroviral plasmids (Fig. 1) were constructed basing on a synthesized plasmid pJET-SAchSTpac (AITbiotech) and pGT-GFP generously provided by Dr. Thijn R. Brummelkamp (The Netherlands Cancer Institute, Amsterdam, Netherlands), which has been reported efficient for virus packaging and gene trapping in near haploid human cells (Carette et al., 2009, 2011a,b; Reiling et al., 2011). The synthesized pJET-SAchSTpac plasmid contained a gene trapping cassette (a strong splicing acceptor (SA) upstream cherry gene (ch)), SV40 polyadenylation signal (polyA) and puromycin N-acetyltranferase (pac) gene driven by SV40 early gene enhancer plus thymidine kinase promoter (ST) for selection. For replacement of pac with pf (pac fused with gfp), PCR was performed to amplify backbone of pJET-pSAchSTpac discarding pac. Then, pf fragment was amplified from pCVpf plasmid and inserted into the backbone fragment without pac by SpeI and BamHI sites, generating pJET-SAchSTpf. Similar strategy was taken to generate pJET-SAchCVpf after insertion of CVpf (CMV promoter + pf) fragment into the backbone discarding STpac. pJET-SAchCVgfp was generated by replacing pf with gfp (amplified from pCVpf) into pJET-SAchCVpf using BamHI and HindIII. Finally, In-Fusion® HD Cloning Kit (Clontech) was used to construct pSAchSTpf/pSAchCVpf/pSAchCVgfp, following the manufacturer's instruction. Briefly, fragments of SAchSTpf/SAchCVpf/SAchCVgfp were amplified from corresponding plasmids and infused with the linearized pGT-GFP backbone by ClaI and BamHI. All primers for PCR during plasmid construction were listed in Supplementary Table 1S.

2.2. Cell culture

Lenti-X 293T cell was purchased from Clontech and maintained at 37 °C under 5% CO_2 in suggested completed medium: 90% Dulbecco's Modified Eagle's Medium (DMEM) with high glucose (4.5 g/l), 4 mM L-glutamine, 3.7 g/l sodium bicarbonate (Sigma-Aldrich) and 10% fetal bovine serum. Medaka haploid cell (HX1A3) was maintained in ESM4 at 37 °C under ambient air as previously described (Yi et al., 2009, 2010; Yuan et al., 2013).

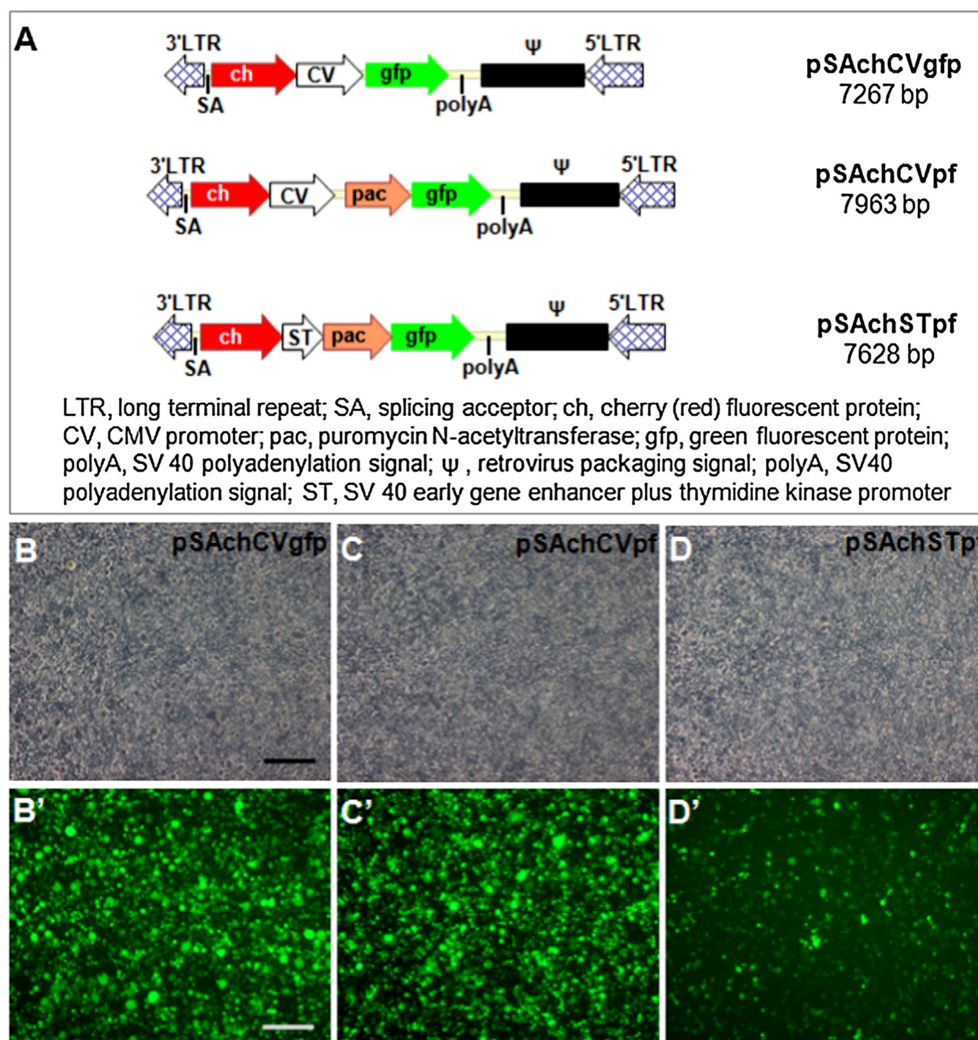

Fig. 1. Retroviral plasmid maps for IM and transfection in 293T cells. A) Plasmid maps for pSAchCVgfp, pSAchCVpf and pSAchSTpf; B–D) phase contrast view of 293T cells transfected with different retroviral plasmids at 48 hpt, respectively. B′–D′) GFP expression of 293T cells transfected with different retroviral plasmids at 48 hpt, respectively. Scale bars, 200 μm.

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