Construction of an inducible lentiviral-based miRNA over-expression library
(Contributed by C6 RNAi Core)

Specific Aim

The overall objective of this project was to develop and construct human and mouse inducible miRNA libraries as new RNAi reagents for the functional genomics studies in the interest of the Taiwan's scientific community.

Introduction

A class of small RNA molecules termed microRNAs (miRNAs) play a pivotal role in development as well as in balancing normal physiology in multicellular organisms (Ambros, 2004; He and Hannon, 2004). The expression of miRNA is frequently deregulated during tumorigenesis, and some miRNAs are involved in cell proliferation and survival (Dalmay and Edwards, 2006). A body of evidence indicates that the altered expression of miRNAs in cancer may involve either loss or amplification of genomic sequences (Dalmay and Edwards, 2006). Thus, miRNAs may function as tumor suppressors or oncogenes. A genetic screen using miRNA library demonstrated that miRNA-372 and miRNA-372 function as oncogenes possibly through direct inhibition of the expression of tumor suppressor LATS2 (Voorhoeve et al., 2006). In a word, miRNA library is useful for identification of potential oncogenes in primary cells and potential tumor suppressor genes in cancer cells. This information prompts us to construct human and mouse inducible miRNA libraries which may benefit Taiwan's scientific community.

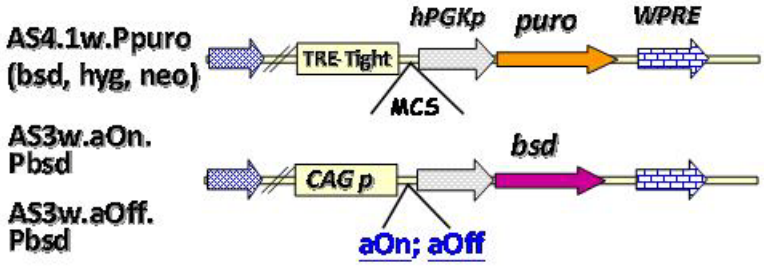
Experimental Design and Results

The precursor of miRNAs (pri-miRNA) is typically transcribed by RNA polymerase II, and transcripts are capped and polyadenylated (Kim, 2005). Pri-miRNAs are processed into pre-miRNAs, a stem-loop structure RNA molecule, by Drosha and cofactor DRCR8 in nucleus. Pre-miRNAs are then exported from the nucleus to cytoplasm where they are further processed by Dicer to form the mature miRNA. Accumulated data indicate that approximately 300-base sequences information of upstream and downstream of the embedded miRNA sequences on pri-miRNA are required for proper processing of the engineered sequences into pre-miRNA. In addition, we thought that inducible system may be more convenient in terms of maintaining the transduced cells. Therefore, we decided to construct a tet-inducible, lentiviral-based miRNA library.

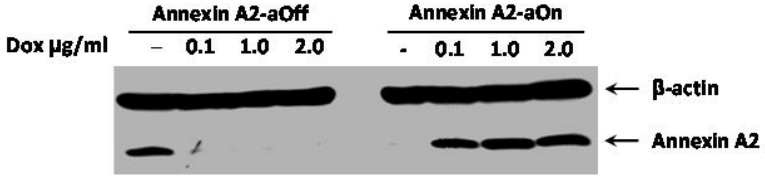
To gain a lentiviral-based inducible system, two kinds of vectors were constructed. (i) The cDNA lentiviral transfer vectors based on tet-inducible system: The tet-inducible vectors were modified from pLKO.1 lentiviral transfer vectors. It contains human PGK (pPGK) expression cassette directing expression of selection markers viz. puromycin (puro), blasticidine (bsd), hygromycin (hyg) or neomycin (neo/G418), respectively. Further these vectors are coupled with the new version of the TRE-tight promoter followed by the multiple cloning sites (mcs) for insertion of the gene/sequence of interest. These vectors can directly be used to prepare VSV-G pseduotyped

lentivirus by following the standard protocol. Resulting virus(es) in combination with advanvced On (aOn) or advanced Off (aOff) lentivirus (see item ii below) provides a powerful tool for successful establishment of an inducible system. (ii) The aOn and aOff-expressing lentiviral vectors. The aOn activates transcriptional activation of the TRE in the presence of doxycycline, an inducer of tet operator. The aOff, on the other hand, inactivates its activation in the presence of doxycycline. The expression cassette was also engineered on lentivviral transfer vector with blasticidin (bsd) selection marker. Thus aOn- or aOff- expressing stable cell line could be established easily by transducing cells with this lentivirus followed by selection against blasticidin.

Their maps are as follows:



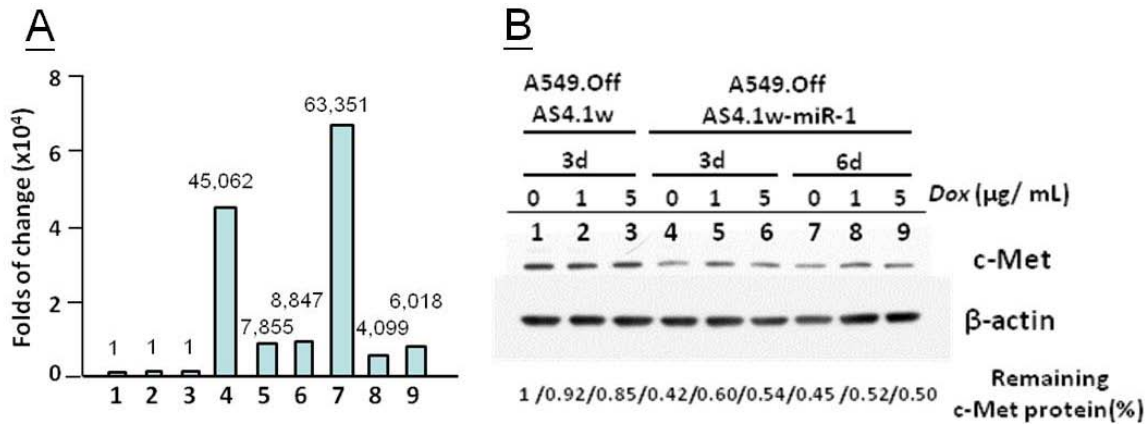
To test the responsibility and tightness of these systems, HEK293 cells were first transduced with AS3w.aOn.Pbsd or AS3w.aOff.Pbsd lentiviruses followed by selecting with blasticidin for 10 days. Resistant cells were then transduced with lentivirus vector expressing Annexin A2 (on pAS4.1.Ppuro vector) followed by selecting with puromycin for three days. As shown in the following figure,



Annexin A2 was not detectable under non-induction conditions as demonstrated by adding doxicyclin in aOff system or without adding doxicyclin in aOn system. On the other hand, the amounts of Annexin A2 gave rise to a promising level after induction. Together, these data indicate that the lentiviral-based inducible system is promising for constructing miRNA library in terms of responsibility and tightness of the inducible system.

To facilitate the construction of miRNA library, we bought a set of CMV promoter-based miRNA expression constructs as templates/inserts for re-cloning them to target lentiviral transfer vector. These miRNA constructs contain 600-650 bps sequences which are needed for proper miRNA processing in vivo. To test whether this design can sustain the expression of authentic miRNA in transduced cells, a miR-1 non expression cell (advanced off transactivator expressing A549 cells) was transduced with AS4.1w-miR-1.As shown in below figure, the miR-1 was indeed expressed in transduced cells as measured by ABI RT-qPCR kit (figure A). Interestingly, C-Met (one of the miR-1 targets) were accordingly downregulated as compared with the control cells (figure B,

A549_AS4.1w). These results suggest that this design can support the expression of miRNA in transduced cells.



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