Tumor suppressors function as a bottleneck against cellular reprogramming into iPS cells

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Hypothesis

It has been shown that a combination of four factors, such as c-Myc, Oct-4, Sox-2, and Klf-4, or Oct-4, Sox-2, Nanog and Lin28, is required for reprogramming of differentiated cells into pluripotent stem cells.

However, reprogramming efficiency appears to be very low (<0.5%), suggesting that genes that are expressed in differentiated cells inhibit reprogramming.

Therefore, I hypothesized that tumor suppressor genes could suppress reprogramming of differentiated cells into iPS cells.

Results

TA-p73 increases the expressions of TRIM32, Ago, and miR-134

- C-terminal NHL domain of TRIM32, an ubiquitin ligase, binds to Argonaute 1(Ago-1, a component of RNA information silencing complex) and thereby the complex TRIM32-Ago1 promotes the efficiency of processing of microRNA-134(figure 1).
- Bioinformatics analysis of m/hTRIM32 promoter revealed more than five p73/p63 binding sites in the TRIM32 promoter, indicating that it could be a transcriptional target of TA-p73/p63 (Table 1). This data suggests a possibility that the TA-p73/p63, by increasing the expression of TRIM32, it could increase the Ago1/2 activity and thereby it could increase the processing of miR-134.

Bioinformatics analysis of miR-134 targets suggests that it could inhibit the expression of

a) Nanog;

b) Sox-2;

c) Oct-4;

and d) LRH1.

This data suggests that TA-p73/p63 could down regulate the reprogramming factors, such as Nanog, Sox-2, Oct-4, and LRH-1, by increasing the expression of miR-134.

In addition, TA-p73 could decrease the expression of stem cell factors, such as Nanog, Sox-2, Oct-4, Klf-4, and c-myc, by increasing the expression of miR-145 (figure 1).

TA-p73/p63 increases the expression of the tumor suppressor let-7 and decreases the expression of lin-28

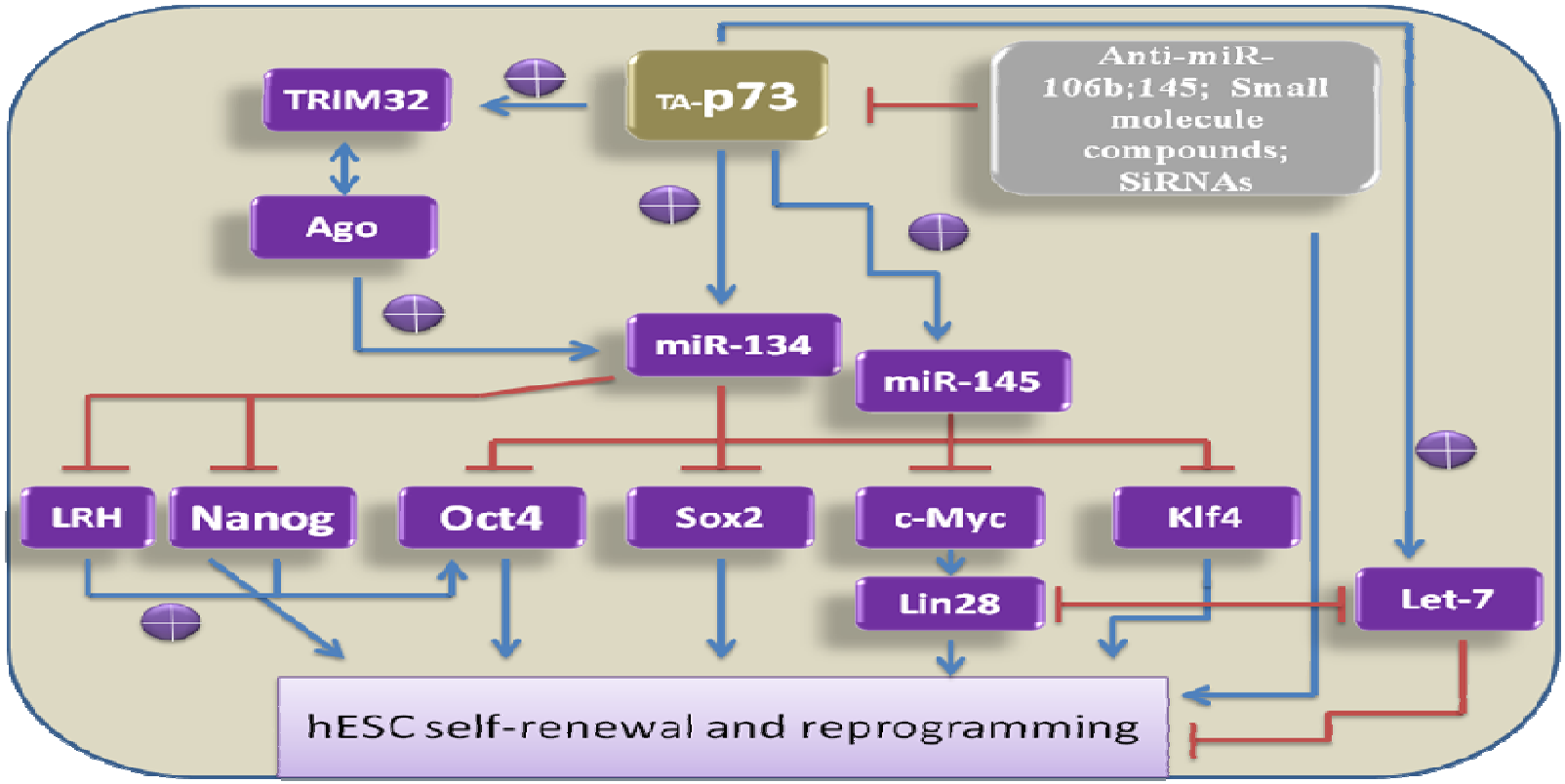
Furthermore, p53/TA-p73/p63 may increase the expression of the tumor suppressor let-7, which, in turn, inhibits the stem cell factor lin-28 expression to inhibit reprogramming. Therefore, inhibiting the expression of

the tumor suppressor TA-p73/p63 will be useful in improving reprogramming efficiency.

Inhibiting the expression of TA-p73/p63 through anti-miR-106b/145 may increase reprogramming

Remarkably, miR-106 appears to increase the expression of TA-p73 by suppressing its negative regulator, Itch, suggesting that by inhibiting the expression of TA-p73/p63 through anti-miR-106, one could increase the efficiency of reprogramming. In addition,

reprogramming efficiency can be improved by transducing the expression vectors containing anti-miR-145 (figure 1).



Human TRIM32 promoter encoades p63/p73 binding sites

Position	TRIM32(h) promoter
-54 to -84	**(tagcaggaat)**ttgaccctcta(**gggcatgaat)**
-840 to -887	**(ttcctaggcc)**ccc(**aaacatgaca)**caataaactctg(**tgacatgatt)**
-3335 to -3357	**(ttacaagttg)**gc(**agccttgttt)**
-4310 to -4330	**(gtgctagaag)(aggcgagatt)**

-4737 to- 4788	**(aagcatggca)**gctttggggcagga (**attcttgaca)**tcctcaga(**gagcttggtg)**

mouseTRIM32 promoter encoades p63/p73 binding sites

Position	TRIM32 promoter
-10 to -41	(**tgccatggga)**a(**ggacgtgcta)(gcgcatgcgc)**
-57 to -79	**(gggcaagttg)**ac(**gggcaggcga)**
-1020 to -1032	(**ggccagggat)**aa(**aatcatgtga)**
-1223 to 1243	(**tctctgggaa)(agccttggga)**

-2949 to -2972	**gttcatgcta**)agc(**tcacaaggct**)
-4375 to -4395	**(ctccttgttc)(tccgaggat)**
-4399 to -4419	(**ggcctggtaa**)(**cacttgact**)
-4468 to -4488	(**aatcaaggtc**)(**tgcatggtg**)
-4565 to -4590	**(ctccaagaga)**tcaga(**caacgaggct**)

Table 1. Bioinformatic analysis of human/mouse TRIM32 promoter sequence for potential p73/p63 binding sites (CWWG). The p63/p73 binding sites are underlined. Mismatching sequences are highlighted in red.

Conclusion

Analysis of microarray data with bioinformatics and computational biology approach revealed that p53/TA-p73/p63-microRNAs could disrupt the core-reprogramming network in ES cells and thereby they could inhibit self-renewal and reprogramming. Therefore, this study suggests that eliminating the tumor suppressor

homologue TA-p73/p63 function is indispensable for improving reprogramming efficiency.