行政院國家科學委員會專題研究計畫 期中進度報告

siRNA 調控 CD94 之胚胎幹細胞模式: 自然殺手細胞淋巴腺癌之病理機轉(2/3)

期中進度報告(精簡版)

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計 畫 主 持 人 ：許世明

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中 華 民 國 96 年 05 月 25 日

行政院國家科學委員會補助專題研究計劃期中進度報告

siRNA 調控 CD94 之胚胎幹細胞模式:自然殺手淋巴腺癌之病理機轉

計畫類別： ■ 個別型計畫 □整合型計畫
計畫編號： NSC 95-2320-B-002 -038 -
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計畫主持人： 許世明
共同主持人： 林中梧
計畫參與人員：

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執行單位：國立台灣大學醫學院病理學研究所

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Main report

Sinonasal lymphoma (SNL) is an EBV-associated NK cell lymphoma endemic in Taiwan. We have previously shown that a restricted killer immunoglobulin-like receptor (rKIR) repertoire without a monoclonal T-cell receptor (mTCR) rearrangement supports a mature NK-cell origin **(Am J Path, 2001, 159, 1671),** and that the presence of CD94 transcripts implies a better prognosis in SNL **(Blood, 2003, 102, 2623).** Recently, it was shown that CD94 transcription is under the control of both a proximal (CD94 1B) promoter and a distal (CD94 1A) promoter (Lieto LD, et al. **J immuno, 2003, 171, 5277)**. Based on this finding, we found that CD94 1A transcripts characterize a lymphoblastic lymphoma (LBL) of immature NK-cell origin with distinct clinical features **(Manuscript submitted).** CD94 thus appears to be a critical determinant in both the diagnosis and prognosis of NK-cell lymphoma. Loss of CD94 1B implies a poor prognosis in SNL, and persistence of CD94 1A is characteristic of NK-LBL.

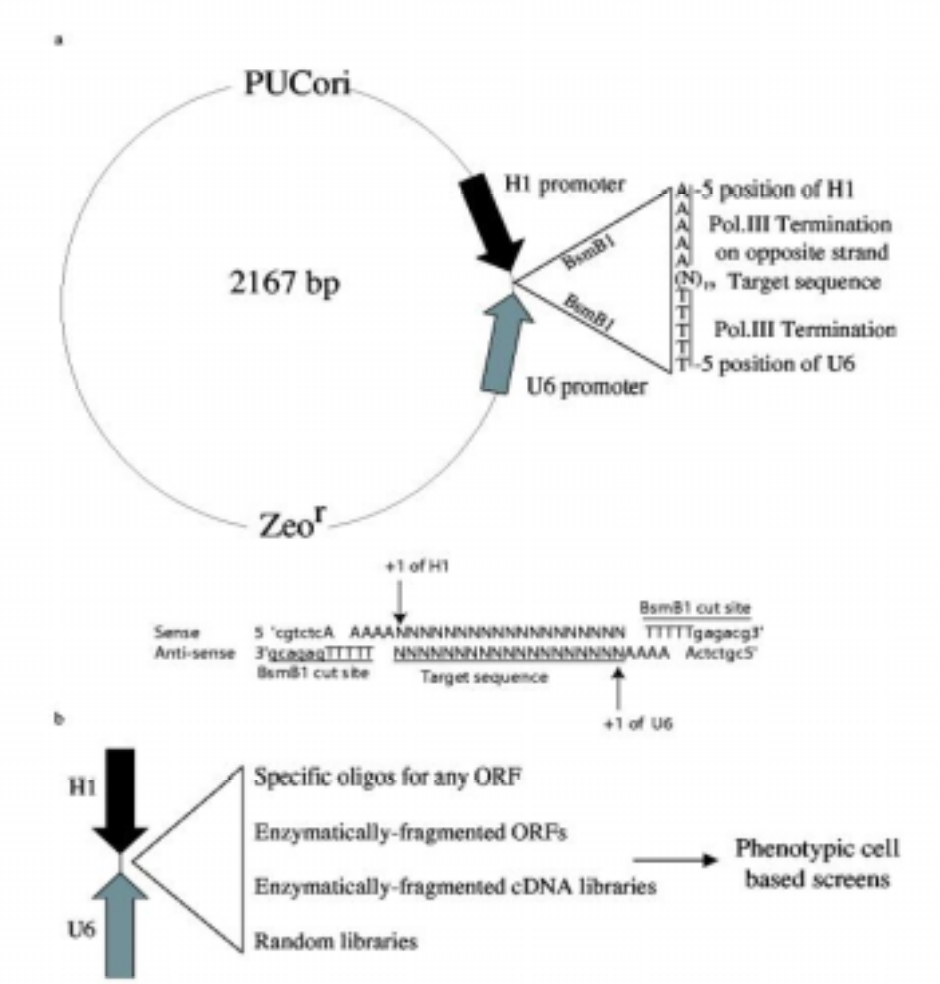
Since microRNAs are known to affect the development of hematopoietic cells (**MicroRNAs modulate hematopoietic lineage differentiation. Science. 2004, 303:83**), we will apply a novel microRNA/RNAi technology (**The RNAi revolution. Nature 2004, 430, 161**) to the study of CD94 transcription.

In the past year, we have completed:

1) a novel microRNA/RNAi plasmid with dual RNA polymerase III promoters
2) construct a comprehensive library for all the known microRNAs in the database,
3) transfect of the library into NK cells and screen for modulation of CD94 transcription

Hopefully, our system will lead to a better undersdtanding of CD94 transcripts in the molecular pathogenesis of SNLs and NK-LBLs.

1) construction of a plasmid with dual promoters



We have constructed a plasmid, modified from the one shown above. This enables us to express microRNAs in an efficient way.

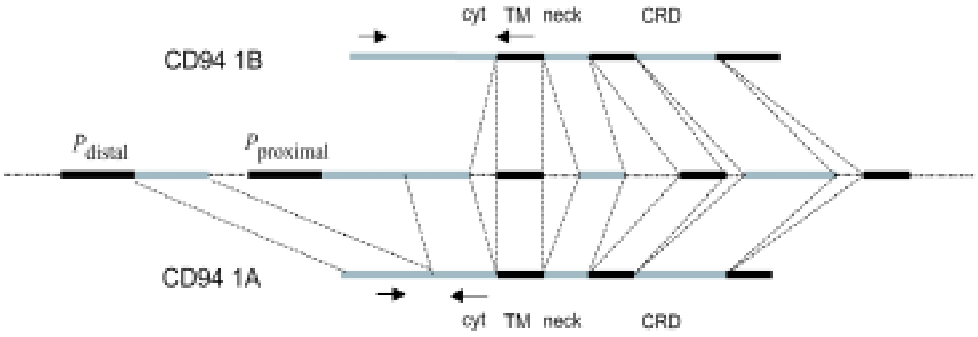
2) We have cloned into the plasmids at least 20,000 clones.
Representative sequences are shown below.

-TGC-CCG-GAC-TCC-CGG-TCG-CTA-
-GCG-GGC-TTG-AAT-TTC-GAA-GAC-
-CTG-AAT-GGA-CAA-CCG-TCC-CTC-
-CGT-GGG-GCT-ACG-CCT-GGG-AAC-
-ATC-AGC-TTT-GGT-GCA-TTG-AAT-
-CGT-GGT-AGA-CGC-CGG-ACT-GAG-
-TTC-CAT-GTA-TGG-CCT-GAA-GTA-*
-AGG-GTC-TTT-ACA-GTA-ACG-AAT-
-AGG-GCT-CCC-TGG-TGG-CCC-AGA-

3)We have successfully transfected the plasmids in NK cell lines.

In the original proposal, we have data only on fibroblasts and lymphoma cell ines of Hodgkin disease. We now are also able to put plasmids into YT cell line, a lymphoma cell line of NK cells. The transfection efficiency is up to 30%. We are also trying another NK cell line, NK-92.

4) We are currently in the middle of construction of a CD94 expression vector.



We are trying various combinations of the promoter and the exons of CD94 to obtain a suitable plasmid for assay of the CD94 promoter activity.

Conclusion & Prospectives:

We have obtained a rather comprehensive microRNA library. We are now actively screening the library for CD94 specific microRNAs. Hopefully, the data will contribute to our understanding not only of CD94 transcription regulation during the development of NK cells, but also the role of micoRNA in hematology and immunology in general (ref 1-4).

Reference

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