論　文　内　容　の　要　旨
Synopsis of Thesis

氏　　名 Name	WIBOWO TANSRI
論文題名 Title	CAR-NK cells derived from cord blood originate mainly from $CD56^-CD7^+CD34^-HLA\text{-}DR^-Lin^-$ NK progenitor cells（臍帯血由来のCAR-NK細胞は主に$CD56^-CD7^+CD34^-HLA\text{-}DR^-Lin^-$ NK前駆細胞から誘導される）

論文内容の要旨

〔目　的(Objective)〕

Cord blood (CB)–derived chimeric antigen receptor (CAR)–natural killer (NK) cells targeting CD19 have been shown to be effective against B-cell malignancies. While human $CD56^+$ NK cells can be expanded in vitro, NK cells can also be differentiated from hematopoietic progenitor cells. It is still unclear whether CAR-NK cells originate from mature NK cells or NK progenitor cells in CB. In this study, we aimed to clarify this issue to improve methods for generating CAR-NK cells from CB cells.

〔方法ならびに成績(Methods/Results)〕

$CD3^-CD56^+$ NK cells or $CD3^-CD56^-$ cells were purified by FACS and separately subjected to 2-week culture using K562 based feeder cells to generate NK cells. We found that substantial numbers of CD19 CAR-NK cells were produced from $CD56^-$ CB mononuclear cells after in vitro culture for 2 weeks. Both $CD56^+$ cell–derived NK cells and $CD56^-$ cell–derived NK cells showed significant cytotoxicity upon co-culture with $CD19^+$ B-cell lymphoma cells. $CD56^-$ cell–derived NK cells showed a greater proliferative potential in response to repeated antigen stimulation compared with $CD56^+$ cell–derived NK cells.

Single-cell RNA sequencing analysis of $CD56^-CD3^-CD14^-CD19^-$ CB mononuclear cells revealed that these cells could be subdivided into three subpopulations based on the expression of CD34 and HLA-DR. These three populations were purified and separately cultured to generate NK cells. After 14 d of culture, NK cells were produced almost exclusively from $CD34^-HLA\text{-}DR^-$ cells.

Single-cell RNA sequencing analysis showed that CD7-expressing cells were almost exclusively detected in the $CD34^-HLA\text{-}DR^-$ cell population, which was subdivided into $CD7^+$ and $CD7^-$ populations. In addition, among several cell surface antigens that were reported to be expressed on NK cells or NK progenitor cells, CD7 expression could be used to subdivide $CD34^-HLA\text{-}DR^-CD56^-Lin^-$ cells into 2 distinct subpopulations, which were then separately cultured to generate NK cells. NK cells were produced from $CD7^+$ cells, but not from $CD7^-$ cells.

〔総　括(Conclusion)〕

$CD56^-CD7^+CD34^-$ $HLA\text{-}DR^-$ lineage marker $(Lin)^-$ cells are the major origin of human CB-derived CAR-NK cells, indicating the importance of developing methods to enhance the quality and quantity of NK cells produced from these NK progenitor cells.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

リンパ腫瘍に対する臍帯血由来CAR-NK細胞療法の有効性と安全性はすでに報告された。臍帯血単核球の中にあるNK細胞がCAR-NK細胞の元となると思われているが、NK細胞以外の細胞からもNK細胞が誘導され、CAR-NK細胞が作られるのかは明らかになっていなかった。この論文によって臍帯血由来CAR-NK細胞は56^{+}NK細胞からのみでなく、$CD56^{-}$の細胞からも誘導され、しかも臍帯血由来CAR-NK細胞はほとんど$CD56^{-}$由来であることが示された。$CD56^{-}$由来CAR-NK細胞は$CD56^{+}$由来のものとほぼ同じPhenotype・Killing機能を示しているが、反復刺激に対しより高い増殖のポテンシャルをもつことも示唆された。$CD56^{-}$分画の中にCAR-NKのもととなる細胞は$Lin^{-}CD34^{-}HLA\text{-}DR^{-}CD7^{+}$のフェノタイプをもつことも同定された。臍帯血由来CAR-NK細胞の作成において$Lin^{-}CD34^{-}HLA\text{-}DR^{-}CD7^{+}$細胞の重要性を初めて主張するものとなり、学位の授与に値すると考えられる。