CHAPTER I
INTRODUCTION

1.1 Background and significance

In recent years, adoptive cell therapy has arisen as an attractive therapeutic option to treat advanced refractory or unresectable cancers. Natural killer cells (NK cells) are cytotoxic lymphocytes that play important roles in early defense against viral infections and tumor surveillance. Without any priming or prior sensitization, NK cells exert its cytolytic activity against viral infected cells and tumor cells via difference mechanisms: direct cytotoxicity via releasing of lytic granules containing perforin and granzymes, activation of antibody-dependent cell-mediated cytotoxicity (ADCC), induction of death receptor-mediated apoptosis via binding of Tumor Necrosis Factor-related apoptosis-inducing ligand (TRAIL) or Fas ligand (FasL) on target cells, and secreting inflammatory cytokines (interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α)) (Wang *et al.*, 2020). Moreover, compared with cytotoxic T lymphocytes (T cells), NK cells take an unique advantage as they do not cause graft versus host disease (GvHD) in allogenic stem cell transplantation (Gill *et al.*, 2009). As a result, adoptive transfer of expanded NK cells is on the way to become the new standard of care of hematological malignancies and some solid tumors (Oh *et al.*, 2019). In order to obtain therapeutic cell dose of NK cells, different expansion methods have been developed. These techniques include, but not limited to, feeder cell-based techniques (Cho *et al.*, 2009; Fujisaki *et al.*, 2009; Granzin *et al.*, 2016), plasma membranes particles or particle-based method (Oyer *et al.*, 2016), stromal support (Oyer *et al.*, 2016), and cytokine-based techniques (Decot *et al.*, 2010; Koehl *et al.*, 2005; Masuyama *et al.*, 2016; Spanholtz *et al.*, 2010).

Since lymphopenia is a well-known side effect of the traditional anticancer treatment, it is, however, not always possible to expand clinically relevant doses of NK cells from heavily treated patients (Young *et al.*, 2019). To overcome this problem, several studies have reported the feasible use of cord blood (CB) as an

alternative source of NK cells (reviewed in Zhao *et al.*, 2020). Moreover, despite its worldwide availability, cord blood-derived NK cells (CBNK cells) have been shown to have a better bone marrow homing ability when compared with peripheral blood derived NK cells (Zhao *et al.*, 2020). So far, CBNK expansion rely on the use of either artificial antigen-presenting (aAPC or feeder) cell-based techniques or cytokines-based techniques. However, in addition to its effectiveness, the presence of aAPC or feeder cells in the culture system open up an opportunity to introduce undesirable cell type into the final product (Halme *et al.*, 2006). For this reason, cytokines-based techniques are more applicable. Cytokines-based techniques employ the use of interleukin (IL)-2 either alone (Xing *et al.*, 2010) or in combination with cytokine cocktail (Spanholtz *et al.*, 2010), tacrolimus and dalteparin sodium (Tanaka *et al.*, 2012), bisphosphonate zoledronic acid (Ma *et al.*, 2018), and group A streptococcus (Mu *et al.*, 2019). Although satisfy number of expanded CBNK cells have been reported from some of these studies, no one has yet come up with known NK cells stimulatory agents: Notch signaling activator, and lipopolysaccharide (LPS). Early studies have shown that activation of Notch signaling promote NK cell differentiation from $CD34^{+}$ hematopoietic stem cells (HSCs) (Benne *et al.*, 2009; Haraguchi *et al.*, 2009). Apart from Notch signaling activator, It is demonstrated that LPS provide beneficial effects on the proliferation (Goodier *et al.*, 2000) and functional activation (Kanevskiy *et al.*, 2019) of peripheral blood derived NK cells (PBNK cells).

In this study, purified functional NK cells were expanded from cord blood derived mononuclear cells (CB-MNCs) using feeder-free and cell-sorting-free approaches. This protocol would pave the way to establish a sustainable supply of NK cells for clinical applications.

1.2 Research objectives

1.2.1. To promote CBNK cell proliferation by stimulating unsorted CB-MNCs with LPS or Yhhu-3792.

1.2.2 To set-up large-scale expansion protocol for CBNK cell production.

1.3 References

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