

# Production of dendritic cells and cytokine-induced killer cells from banked umbilical cord blood samples

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**Abstract**— Umbilical cord blood (UCB) is considered to be a source of hematopoietic stem cells (HSCs). All UCB banks have recently become interested in the isolation and storage of HSCs for the treatment of hematological diseases. However, UCB was also recently confirmed as a source of immune cells for immunotherapy such as dendritic cells (DCs) and cytokine-induced killer cells (CIKs). This study aimed to exploit this source of immune cells in banked UCB samples. After collection of UCB samples, mononuclear cells (MNCs) containing stem cells, progenitor cells, and mature cells were isolated by Ficoll-Hypaque-based centrifugation. The MNCs were subjected to freezing and thawing according to a previously published protocol. The banked MNCs were used to produce DCs and CIKs. To produce DCs, MNCs were induced in RPMI 1640 medium supplemented with GM-CSF (50 ng/ml) and IL-4 (40 ng/ml) for 14 days. To produce CIKs, MNCs were induced in RPMI 1640 medium supplemented with anti-CD3 monoclonal antibody, IL-3, and GM-CSF for 21–28 days. Both DCs and CIKs were evaluated for their phenotypes and functions according to previously published protocols. The results showed that banked UCB samples can be successfully used to produce functional DCs and CIKs. These samples are valuable sources of immune cells for immunotherapy. The present results suggest that banked UCB samples are useful not only for stem cell isolation, but also for immune cell production.

**Keywords**—Banked UCB, Stem cells, Immune cells, Dendritic cells, Cytokine-induced killer cells, Immunotherapy

## INTRODUCTION

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## RESULTS

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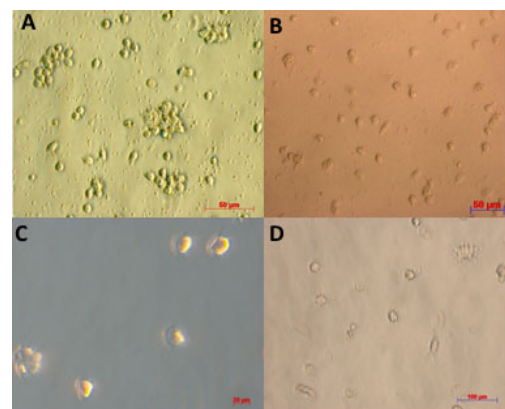
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**Figure 1. Banked MNCs were successfully induced into dendritic cells.** MNCs were thawed from cryoprecipitated samples (A), were induced into dendritic cells after 7 days (B) and 14 days (C, D).

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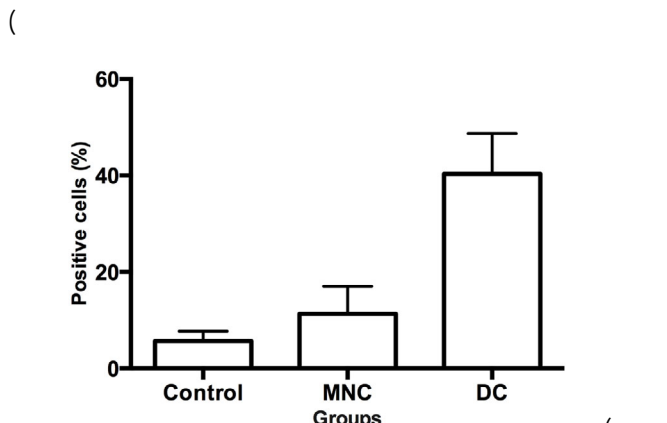


Figure 2. Flow cytometry analysis of DC phagocytosis using FITC-dextran. The induced DCs phagocytose the FITC-dextran compared with control cells.

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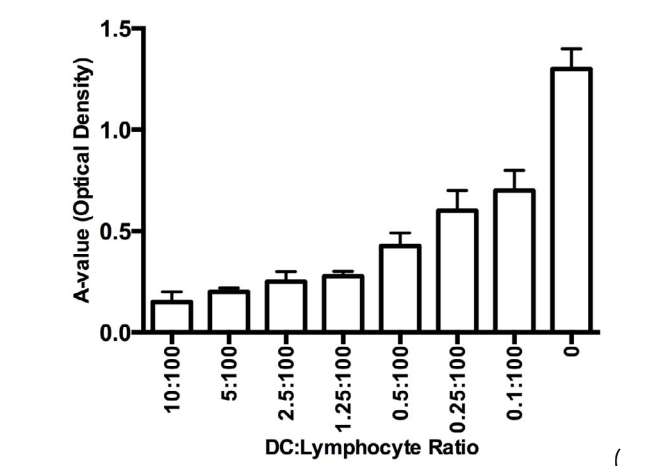


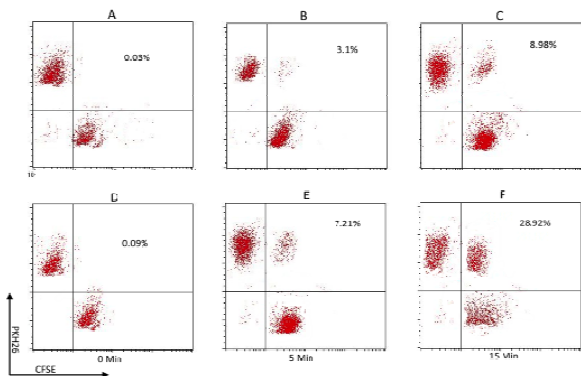
Figure 3. DC candidates stimulate the proliferation of allogenic T cells. DC can stimulate the T proliferation on the ratio of DCs to T cells. The ratio of 1 DC to 10 T cells exhibited the highest stimulating efficacy.

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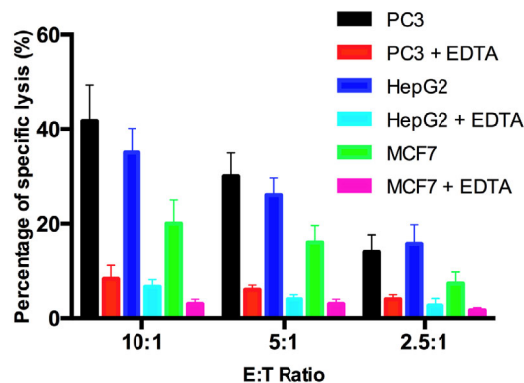
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**Figure 4. CIKs can bind to target cells (MCF7).** In the control group (A-B-C), with EDTA, CIKs also can bind to target cells but with low efficacy; while in the experimental group, CIKs can bind to target and were maximal at 15 min after mixing (D-E-F).

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**Figure 5. CIKs lysed target cells (HepG2, PC3, and MCF7).** CIKs successfully lysed the target cells in a manner dependent on the E:T ratio. CIKs could lyse all three kinds of cancer cells (HepG2, red; PC3, blue; MCF7, black). In the control group, EDTA was added to prevent binding of CIKs to the target cells, and the percentage of lysis was very low (white).

## DISCUSSION

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## CONCLUSIONS

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## Abbreviations

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## Acknowledgement

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## Competing interests

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## References

- Bracho, F., Van De Ven, C., Areman, E., Hughes, R., Davenport, V., Bradley, M., Cai, J., and Cairo, M. (2003). A comparison of ex vivo expanded DCs derived from cord blood and mobilized adult peripheral blood plastic-adherent mononuclear cells: decreased alloreactivity of cord blood DCs 1. *Cytotherapy* 5, 349-361.
- Dalle, J.H., Menezes, J., Wagner, E., Blagdon, M., Champagne, J., Champagne, M.A., and Duval, M. (2005). Characterization of cord blood natural killer cells: implications for transplantation and neonatal infections. *Pediatr Res* 57, 649-655.
- Durrieu, L., Dieng, M.M., Le Deist, F., and Haddad, E. (2013). Cord blood-derived and peripheral blood-derived cytokine-induced killer cells are sensitive to Fas-mediated apoptosis. *Biol Blood Marrow Transplant* 19, 1407-1411.
- Enomoto, M., Nagayama, H., Sato, K., Xu, Y., Asano, S., and Takahashi, T. (2000). In vitro generation of dendritic cells derived from cryopreserved CD34+ cells mobilized into peripheral blood in lymphoma patients. *Cytotherapy* 2, 95-104.
- Fan, Y.Y., Yang, B.Y., and Wu, C.Y. (2008). Phenotypic and functional heterogeneity of natural killer cells from umbilical cord blood mononuclear cells. *Immunol Invest* 37, 79-96.
- Gluckman, E. (2009). History of cord blood transplantation. *Bone Marrow Transplant* 44, 621-626.
- Gluckman, E., Broxmeyer, H.A., Auerbach, A.D., Friedman, H.S., Douglas, G.W., Devergie, A., Esperou, H., Thierry, D., Socie, G., Lehn, P., et al. (1989). Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N Engl J Med* 321, 1174-1178.
- Hanley, P.J., Cruz, C.R., Shpall, E.J., and Bollard, C.M. (2010). Improving clinical outcomes using adoptively transferred immune cells from umbilical cord blood. *Cytotherapy* 12, 713-720.
- Introna, M., Franceschetti, M., Ciocca, A., Borleri, G., Conti, E., Golay, J., and Rambaldi, A. (2006). Rapid and massive expansion of cord blood-derived cytokine-induced killer cells: an innovative proposal for the treatment of leukemia relapse after cord blood transplantation. *Bone marrow transplantation* 38, 621-627.

Kim, S.K., Yun, C.H., and Han, S.H. (2015). Dendritic Cells Differentiated from Human Umbilical Cord Blood-Derived Monocytes Exhibit Tolerogenic Characteristics. *Stem Cells Dev.*

Kurtzberg, J., Laughlin, M., Graham, M.L., Smith, C., Olson, J.F., Halperin, E.C., Ciocchi, G., Carrier, C., Stevens, C.E., and Rubinstein, P. (1996). Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. *N Engl J Med* 335, 157-166.

Li, Y., Schmidt-Wolf, I.G., Wu, Y.-F., Huang, S.-L., Wei, J., Fang, J., Huang, K., and Zhou, D.-H. (2010). Optimized protocols for generation of cord blood-derived cytokine-induced killer/natural killer cells. *Anticancer research* 30, 3493-3499.

Liu, Y., Tian, X., Jiang, S., Ren, X., Liu, F., Yang, J., Chen, Y., and Jiang, Y. (2015). Umbilical cord blood-derived dendritic cells infected by adenovirus for SP17 expression induce antigen-specific cytotoxic T cells against NSCLC cells. *Cell Immunol.*

Niu, Q., Wang, W., Li, Y., Qin, S., Wang, Y., Wan, G., Guan, J., and Zhu, W. (2011). Cord blood-derived cytokine-induced killer cells biotherapy combined with second-line chemotherapy in the treatment of advanced solid malignancies. *Int Immunopharmacol* 11, 449-456.

Park, Y.S., Shin, C., Hwang, H.S., Zenke, M., Han, D.W., Kang, Y.S., Ko, K., Do, Y., and Ko, K. (2015). In vitro generation of functional dendritic cells differentiated from CD34 negative cells isolated from human umbilical cord blood. *Cell Biol Int* 39, 1080-1086.

Phuc, P.V., Ngoc, V.B., Lam, D.H., Tam, N.T., Viet, P.Q., and Ngoc, P.K. (2012). Isolation of three important types of stem cells from the same samples of banked umbilical cord blood. *Cell Tissue Bank* 13, 341-351.

Phuc, P.V., Nhung, T.H., Loan, D.T., Chung, D.C., and Ngoc, P.K. (2011). Differentiating of banked human umbilical cord blood-derived mesenchymal stem cells into insulin-secreting cells. *In Vitro Cell Dev Biol Anim* 47, 54-63.

Ruggeri, L., Mancusi, A., Capanni, M., Urbani, E., Carotti, A., Aloisi, T., Stern, M., Pende, D., Perruccio, K., Burchielli, E., et al. (2007). Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. *Blood* 110, 433-440.

Verneris, M.R., and Miller, J.S. (2009). The phenotypic and functional characteristics of umbilical cord blood and peripheral blood natural killer cells. *Br J Haematol* 147, 185-191.

Wang, L., Huang, S., Dang, Y., Li, M., Bai, W., Zhong, Z., Zhao, H., Li, Y., Liu, Y., and Wu, M. (2014). Cord blood-derived cytokine-induced killer cellular therapy plus radiation therapy for esophageal cancer: a case report. *Medicine (Baltimore)* 93, e340.

Wang, Y., Xu, H., Zheng, X., Wei, H., Sun, R., and Tian, Z. (2007). High expression of NKG2A/CD94 and low expression of granzyme B are associated with reduced cord blood NK cell activity. *Cell Mol Immunol* 4, 377-382.

Zhang, Q., Wang, L., Luo, C., Shi, Z., Cheng, X., Zhang, Z., Yang, Y., and Zhang, Y. (2014). Phenotypic and functional characterization of cytokine-induced killer cells derived from preterm and term infant cord blood. *Oncol Rep* 32, 2244-2252.

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