UNIVERSITI TEKNOLOGI MARA

LINEAGE NEGATIVE (lin^-) CELL ISOLATION, DIFFERENTIATION AND CHARACTERIZATION FROM UMBILICAL CORD BLOOD

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ABSTRACT

Among the well-established and mostly studied adult stem cells, umbilical cord blood-derived lineage negative (lin^-) cells were chosen to be studied. The stem cell has been regarded to have many potential applications due to its ability for self-renewal and differentiation. Studies presented here showed that there is still uncertainty on the benefits of using positive markers such as CD34 in isolating the most primitive cells in Umbilical Cord Blood (UCB). By using selective markers, isolation and characterization of lineage negative (lin^-) cells were conducted. A novel negative selection protocol was designed to isolate the lineage negative cells of UCB from 50 mothers with normal full-term deliveries. The UCB samples were lysed using ammonium chloride lyses buffer and then stained with cocktails of monoclonal antibodies. The cells sorting of $CD34^-lin^-$ were conduct by fluorescence-activated cell sorter (FACS). Clonal culture of (FACS) $CD34^-lin^-$ UCB cells revealed proliferation capacity in semi-solid methycellulose media by forming CFU-GM and CFU-E. Currently, the isolated cells were unable to differentiate as true stem cells or very early progenitor cells. However, the study has proved that the isolated lin- UCB cells can be differentiated to B-lymphoid lineage when induced with specific cytokines. B-lymphoid cells were still considered blood cells; the true status of the lin^- cells isolated from UCB is still unclear.

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CHAPTER 1

INTRODUCTION

1.0 Study Background.

Recent years have seen much excitement over the possibility that mammalian stem cells may be capable of differentiating across tissue lineage boundaries and such may represent novel, accessible and very versatile effectors of therapeutic tissue regeneration. Stem cells are self-renewing, unspecialized cells that can give rise to multiple types all of specialized cells of the body. The process by which dividing, unspecialized cells are equipped to perform specific functions of differentiation and are fundamental to the development of the mature organism. It is now known that stem cells in various forms can be obtained from the embryo, the fetus and the adult.

1.1 The Problem Statement.

One of the first major events in the embryonic development is the specification of the three embryonic germ layers: ectoderm (believed to give rise to skin and neural lineages), mesoderm (believed to generate blood, bone, muscle, cartilage and fat), and endoderm (believe to contribute tissues of the respiratory and digestive tracts). Their almost limitless potential has made embryonic stem cells a significant focus of medical research. Adult stem cells are found in the heart, brain, bone marrow, lungs and other organs, which were once believed to be limited only to stem cells and give, rise to the same type of tissue from which they originated. But new research suggests that adult stem cells may have the potential to generate other types of cells [1]. An example is the liver cells, which may be coaxed to produce insulin, which are normally made by the pancreas. This capability is known as plasticity or transdifferentiation. Adult and alternative sources of stem cells have demonstrated much brighter prospects. This misperception has societal consequences, distorting the political debate over human cloning and embryonic-stem-cell research (ESCR) and perhaps even affecting levels of public and private research funding of embryonic and adult stem-cell therapies.