RESEARCH PAPER

Organogenesis 8:3, 1–5; July/August/September 2012; © 2012 Landes Bioscience

Efficient stem cell isolation from under vacuum preserved tissue samples

Aldo Moggio,[1] Giuseppe D'Armento[2] and Benedetta Bussolati[1,*]

[1]Department of Internal Medicine; Center for Molecular Biotechnology; Torino, Italy; [2]Department of Biomedical Sciences and Human Oncology; University of Torino; Torino, Italy

Keywords: stem cell culture, CD133, tissue, isolation, methods

Abbreviations: UVSC, under vacuum sealing and cooling; FBS, foetal bovine serum; THP, Tamm-Horsfall protein; H/E, hematoxylin/eosin; EBM, endothelial basal medium

Different approaches for the isolation of stem/progenitor cells have been reported, including stem cell selection in stringent culture conditions. We evaluated the possibility of isolating human progenitor cells from surgical specimens preserved by under vacuum sealing and cooling, a clinical practice approached by several hospitals as alternative to formalin. Renal tissue samples (n = 20) maintained under vacuum from 6 to 48 h at 4°C were used to isolate human renal CD133$^+$ progenitor cells. We obtained CD133$^+$ progenitors from unsorted cells derived from disaggregated tissues from each sample. Phenotypic characterization as well as in vitro and in vivo differentiation of the obtained CD133$^+$ lines showed results comparable with sorted CD133$^+$ cells obtained from fresh tissue. These results indicate that the process of sealing under vacuum and cooling appears as a suitable tissue treatment to isolate hypoxia resistant cells, such as human stem/progenitor cells, and that this procedure can be exploited to render the extraction of stem cells from human samples more practical and feasible.

Introduction

Isolation and culture are crucial techniques for studying stem cell biology and modulation. In particular, in studies on human cells, samples from tissue removed for clinical procedures and discarded by pathologists are commonly utilized as a stem/progenitor source for research studies. However, the routine use of formalin, both as a preserver and fixative for histological processing, may limit the possible use of pathological samples for cell isolation. As formalin is encountering increasing criticisms for toxicity, carcinogenicity and environmental concerns,[1] several hospitals are now approaching the use of fresh tissue sample transfer from surgery to the pathology service.[2] Such transfer, and related "ischemic time" is heavily dependent on local conditions and habits.

In the major university hospital we are related to, transfer of surgical specimens under condition of vacuum sealing and cooling (UVSC) has become a habit for the past 4 years.[2] Merits of this procedure in terms of morphological, immunohistochemical and nucleic acid preservation have already been reported.[3] We have considered that the UVSC procedure may offer advantages for stem cell preservation and culturing as well. In fact, low oxygen tension is an important component of the stem cell microenvironment and niche and it provides signals conducive to the maintenance of definitive stem cell properties.[4,5] We therefore hypothesized that the anoxic conditions of tissue samples under vacuum may allow survival of undifferentiated stem/progenitor cells.

We previously reported on the isolation of CD133$^+$ progenitor cells from normal fresh specimens of human kidney.[6] In the present study, we show the successful isolation of CD133$^+$ cells from 20 renal tissue samples maintained under vacuum from 24 to 48 h at 4°C. The results show in all cases a selective survival of stem cells in the anoxic condition characterizing the vacuum procedure, and show that this approach is suitable for stem cell isolation in terms of feasibility and practice.

Results and Discussion

Different approaches for the isolation of stem/progenitor cells have been reported. A direct method may involve the isolation of stem cells by a known marker, such as CD133,[7] or alternatively by a cell function such as the capacity to efflux Hoechst dye.[8] Negative methods are based on the elimination of unwanted differentiated or contaminating cells. In this regard, selective culture conditions that only allow survival of undifferentiated cells might be used, e.g., by removal of serum and by using plastic dishes that do not support cell adhesion.[9,10] Here, we evaluated UVSC treatment of normal tissues could be useful for selective survival and isolation of human renal CD133$^+$ progenitor cells.

CD133$^+$ progenitor cells are present as a minor population within the renal tubules of the nephron and were previously isolated by immunomagnetic sorting.[6,11] As in tissue undergoing vacuum and cooling the percentage of viable cells was very low

*Correspondence to: Benedetta Bussolati; Email: benedetta.bussolati@unito.it
Submitted: 06/18/12; Revised: 07/26/12; Accepted: 08/11/12
http://dx.doi.org/10.4161/org.21816