Research Article

Stem Cell & Regenerative Medicine

Stage-Specific Embryonic Antigen-4-Positive Cells and Carcinoembryonic Antigen Cell Adhesion Molecule-1-Positive Cells are Located in the Bone Marrow of the Adult Rat

Young HE[1,2,4*], Henson NL[1,5], Black GF[1], Hawkins KC[3], Coleman JA[1] and Black AC Jr[6]

[1]*Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA, USA.*

[2]*Department of Pediatrics, Mercer University School of Medicine, Macon, GA, USA.*

[3]*Department of Obstetrics & Gynecology, Mercer University School of Medicine, Macon, GA, USA.*

[4]*Dragonfly Foundation for Research & Development, Macon, GA, USA.*

[5]*Chief of Radiology, St. Francis Hospital, Columbus, GA, USA.*

[6]*Department of Medical Education, University of South Carolina-Greenville, Greenville, SC, USA.*

[*]Correspondence:
Henry E. Young, Chief Science Officer, Dragonfly Foundation for Research and Development, 1515 Bass Rd, Suite E (Corporate Office), Macon, GA 31210, USA, Tel: 478-319-1983; Fax: 478-743-0280; E-mail: young.hey1@yahoo.com.

Received: 18 July 2017; **Accepted:** 24 July 2017

Citation: Young HE, Henson NL, Black GF, et al. Stage-Specific Embryonic Antigen-4-Positive Cells and Carcinoembryonic Antigen Cell Adhesion Molecule-1-Positive Cells are Located in the Bone Marrow of the Adult Rat. Stem Cells Regen Med. 2017; 1(2) 001: 1-3.

ABSTRACT

Pluripotent stem cells and totipotent stem cells have been discovered within the skeletal muscle of adult mammals, including humans. The proposed hypothesis is that these stem cells are located ubiquitously throughout the body. Thus, pluripotent stem cells and totipotent stem cells should be located within bone marrow of the adult rat. Adult rats were euthanized following the guidelines of Mercer University's IACUC. Bone marrow was harvested and processed via cytospinning prior to staining with antibodies diagnostic for endogenous adult-derived stem cells. Two antibodies were utilized: stage-specific embryonic antigen-4 (SSEA-4) and carcinoembryonic antigen-cell adhesion molecule-1 (CEA-CAM-1). Both SSEA-4-positive cells and CEA-CAM-1-positive cells were present as mixed populations of cells within the bone marrow. Future studies will address their functional significance during tissue maintenance and healing.

Keywords

Totipotent stem cells, CEA-CAM-1, Pluripotent stem cells, SSEA-4, Bone marrow, Mammals, Immunocytochemistry.

Introduction

Embryonic stem cell research has been embroiled in ethical and moral debate for many years. During this time, an intriguing alternative source of naturally-occurring endogenous cells has been identified in postnatal tissue of mammals, including humans. Multiple populations of precursor cells have been identified in various organs of the body. These cells exhibit individual or collective capabilities similar to that of embryonic stem cells [1,2]. These cells hold exciting possibilities for the maintenance and healing of tissues damaged by chronic disease and/or severe trauma [3]. Precursor cells have been identified at all levels of differentiation. These range from precursor cells that are only capable of forming one type of functional cell (such as unipotent progenitor cells) to precursor cells that can form multiple tissue types [3,4]. The latter cells have potentials similar to those of primitive embryonic stem cells. Pluripotent stem cells (PSCs) potentially exist within all the organs of the body and have the ability to form all the somatic cells of the body [5]. Pluripotent stem cells have been identified, isolated, and cloned from single cells. The differentiation potentials of PSCs have been characterized using cell surface markers, telomerase activity, tissue concentration, proliferation activity, culture characteristics, and phenotypic expression markers [2]. One of the more recent types of precursor cells to be isolated and characterized are the totipotent stem cells (TSCs). These cells

resemble the function of embryonic blastomeres with respect to their ability to differentiate into all the cell types within the body, including the germ cells [1]. In addition, TSCs resemble embryonic stem cells in that they express several markers characteristic of these cells [3]. These two populations of cells, TSCs and PSCs, are important for many reasons when compared to further differentiated types such as the germ layer lineage stem cells (ectodermal stem cells, mesodermal stem cells, and endodermal stem cells) and progenitor cells. Totipotent stem cells and PSCs have a faster proliferation rate and higher replicative potential due to their lack of contact inhibition [2,3]. Thus, even though they are found in small concentrations within a given tissue, it is possible to replicate these cells to produce large quantities without losing their differentiation potential. This characteristic could serve as a valuable resource for the development of cellular therapeutics.

The overall purpose of this study was to test the hypothesis that TSCs and PSCs are present in all tissues and organs of the body. The specific goal was to identify TSCs (using antibodies to CEA-CAM-1) and PSCs (using antibodies to SSEA-4) in the bone marrow of adult rats.

Materials and Methods

Animal Use

The use of animals in this study complied with the guidelines of Mercer University. These guidelines reflect the criteria for humane animal care of the National Research Council. These criteria are outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (National Academy Press, 1996).

Harvest of Bone Marrow from Adult Rats

Adult male Sprague-Dawley rats (n=10) were humanely euthanized using carbon dioxide inhalation, as per Mercer University IACUC protocols. Once euthanized, the rats were placed on crushed ice. The Achilles tendon was cut and the skin split with scissors up past the hip. The skin was peeled back past the hip joint and the muscles were cut away from the femur and tibia. The lower extremity was then removed from the hip joint. After the foot was cut away from the ankle, the femur and tibia were separated at the knee joint.

A solution of Dulbecco's Phosphate Buffered Saline (DPBS, GIBCO, Invitrogen Corporation, Carlsbad, CA) containing 2% Fetal Calf Serum (GIBCO) (2% FCS-DPBS) was used to aspirate the bone marrow from the bones. A one milliliter (ml) tuberculin syringe with a 27-Gauge needle was filled with 2% FCS-DPBS. The ends of the bones were carefully clipped with sharp scissors, one at a time, and the bone marrow was expressed into an empty collection tube that had been kept on ice. This was repeated for each of the four long bones of the lower extremity in each rat. The bone marrow from the four bones of each rat was pooled, placed into a tube and labeled appropriately. The rat carcasses were frozen to await incineration according to University protocols. The carbon dioxide euthanasia cage was then cleaned with disinfectant.

The bone marrow samples were kept on ice and vortexed at high speed to disperse any chunks of marrow. Cell counts were performed and calculations determined for the cytospin slides. Approximately 200 µl, containing 2.70 x 10^5 cells, were utilized per slide. The samples were kept on ice and in the refrigerator.

After three hours of refrigeration the cytospins were started. The slides were labeled first and then assembled for the cytospin. The cells were vortexed to ensure a homogenous solution prior to adding to the funnel. The final volume was brought up to 400 µl per slide using DPBS. The slides were spun at 200 x 10 rpm for 5 minutes. The slides were then fixed in 100% methanol for approximately 7 minutes at ambient temperature and allowed to air dry. The slides stayed in a slide box at ambient temperature overnight prior to staining.

Staining Bone Marrow

The next morning, the slides were rinsed with DPBS twice and then inverted to remove excess fluid. They were incubated in 5% sodium azide for 30 minutes at ambient temperature. They were rinsed once with DPBS and inverted to remove the fluid. Next, the slides were incubated for 60 minutes at ambient temperature with a solution of 30% hydrogen peroxide. The sodium azide and hydrogen peroxide were used to irreversibly inhibit any endogenous peroxidases present on the surface of the cells.

While the slides were incubating in the hydrogen peroxide, the antibodies were diluted to proper concentrations. The slides were rinsed twice with DPBS and inverted to remove excess fluid. The primary antibodies were added and incubated for 60 minutes at ambient temperature. The primary antibodies used were CEA-CAM-1 (clone 5.4, Hixson) for totipotent stem cells and SSEA-4 (MC813-70, Developmental Studies Hybridoma Bank, Iowa City, IA) for pluripotent stem cells [4]. 1A4 (Sigma, St Louis, MO) was used as a positive procedural control to stain smooth muscle alpha-actin in the tunica media of the vasculature. Negative procedural controls consisted of: PBS alone, no primary antibody, no secondary antibody (Rat Adsorbed Biotinylated Anti-Mouse IgG, Vector Laboratories Inc., Burlingame, CA) and no tertiary probe (Avidin-HRP, Vector).

The slides were rinsed twice with DPBS and inverted to remove excess fluid. The secondary antibody, rat adsorbed Biotinylated anti-IgG, was added and incubated for 60 minutes at ambient temperature. The slides were rinsed twice with DPBS and inverted to remove excess fluid.

The tertiary probe (Avidin-HRP) was added and incubated at ambient temperature for 60 minutes. The slides were rinsed four times with DPBS and inverted to remove excess fluid. The AEC (Sigma) HRP-substrate solution was prepared, added to the slides, and incubated for 60 minutes at ambient temperature.

The slides were rinsed twice with DPBS and inverted to remove excess fluid. Kim Wipes were used to remove remaining fluid from the slides and the slides cover-slipped with Vecta-Mount (Vector).

Photography and Preparation

The cells were photographed using a COOLPIX 995 digital camera and a Nikon Phase Contrast TMS microscope. The digital photographs were cropped using Adobe Photoshop 7.0.

Results

Clusters of CEA-CAM-1-positive cells (putative TSCs) (Figure 1A) were present in the bone marrow preparation. Individual SSEA-4-positive cells (putative PSCs) (Figure 1B) were also present in the bone marrow preparation, but were fewer in number than the CEA-CAM-1-positive cells. IA4-positive arterioles (Figure 1C) were present in the bone marrow preparation as the positive staining control and the negative controls (Figure 1D) showed absence of any staining.

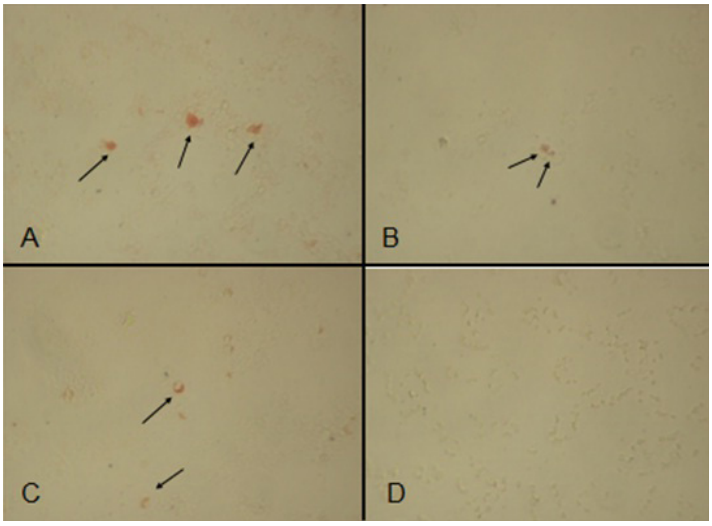

Figure 1: Staining of non-hematopoietic bone marrow cells.
A: Clusters of CEA-CAM-1 positive cells (arrows).
B: Individual SSEA-4 positive cells (arrows).
C: IA4-positive arterioles (arrows) are present in the bone marrow preparation.
D: Representative picture showing absence of staining in the negative controls.

Discussion

In this study we demonstrated that non-hematopoietic stem cells exist in bone marrow of the adult rat. The non-hematopoietic stem cells that were isolated were identified as TSCs and PSCs, utilizing staining for their unique cell surface markers, i.e., CEA-CAM-1 and SSEA-4, respectively [4]. These particular non-hematopoietic stem cells feature the ability to differentiate into varied cell types and have the potential to reconstitute the entire bone marrow population of cells [3-5]. As TSCs and PSCs are normally dormant and only become activated either to proliferate or differentiate under specific cues, Young and colleagues have not seen or reported teratoma formation or uncontrolled cell division as compared to embryonic stem cells [6]. These endogenous adult stem cells may additionally offer specific advantages over embryonic stem cells, as TSCs and PSCs can be isolated from a patient's tissue, expanded in vivo or in vitro, and then administered to the patient in an autologous manner. The use of these stem cells in this manner would theoretically minimize the risk associated with tissue rejection as well as mitigate the need for chronic immunosuppression to prevent graft rejection [7]. Future studies will address the ubiquitous nature of TSCs and PSCs throughout the body, as well as specific experiments to determine their ability to reconstitute previously irradiated bone marrow, using the populations of allogeneic and/or autologous TSCs and PSCs.

Acknowledgements

Funding for this study was provided in part from RESOLVE, MedCen Foundation, Rubye Ryle Smith Charitable Trust, LM & HO Young Estate Trust, and Moraga Biotechnology Corporation. We would like to thank M Carreiro, AM Rice, L Powell, AJ Thompson, K Detmer, and C Alena for their technical assistance. Clone 5.4 of the CEA-CAM-1 antibody was the kind gift of Douglas Hixson, Department of Internal Medicine, Brown University, Providence, RI, 02903, USA. SSEA-4 (MC813-70) developed by D. Solter was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA.

References

1. Young HE, Duplaa C, Katz R, et al. Adult-derived stem cells and their potential for tissue repair and molecular medicine. J Cell Molec Med. 2005; 9: 753-769.
2. Young HE, Black AC. Pluripotent Stem Cells, Endogenous versus Reprogrammed, a Review. MOJ Orthop Rheumatol. 2014; 1: 00019.
3. Young HE, Speight MO, Black AC Jr. Functional cells, maintenance cells, and healing cells. Stem Cells Regen Med. 2017; 1: 1-4.
4. Young HE, Black Jr AC. Adult stem cells. Anat Rec. 2004; 276A: 75-102.
5. Young HE, Duplaa C, Yost MJ, et al. Clonogenic analysis reveals reserve stem cells in postnatal mammals. II. Pluripotent epiblastic-like stem cells. Anat Rec. 2004; 277A: 178-203.
6. Young HE, Black AC Jr. Adult-derived stem cells. Minerva Biotechnologica. 2005; 17: 55-63.
7. Kucia M, Zuba-Surma E, Wysoczynski M, et al. Physiological and pathological consequences of identification of very small embryonic like (VSEL) stem cells in adult bone marrow. J Physiol Pharmacol. 2006; 57: 5-18.
8. Young HE, Cecile Duplaa, Marina Romero-Ramos, et al. Adult reserve stem cells and their potential for tissue engineering. Cell Biochem Biophys. 2004; 40: 1-80.
9. Serafini M, Scott J. Dylla, Masayuki Oki, et al. Hematopoietic reconstitution by multipotent adult progenitor cells: precursors to long-term hematopoietic cells. J Exp Med. 2007; 204: 129-139.