HESCA-1 and HESCA-2: Novel Antibodies for the Identification of Human Embryonic Stem Cells

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Abstract

Pluripotent human embryonic stem cells (hESCs) are a major focus of research because of their experimental utility and great promise in regenerative medicine. However, only a limited number of hESC cell lines have been approved for federally-funded research, and few tools are available for sufficiently defining pluripotent hESCs compared with more differentiated populations. Thus, the need for new monoclonal antibodies against cell surface markers for the further study of stem cells is evident. Using a proprietary platform for selecting hybridomas, we were able to label at least two antibodies that appear to label undifferentiated hESCs and thus may represent novel markers for pluripotency.

Introduction

Embryonic stem cells (ES) are rapidly growing, pluripotent cells with the capacity to differentiate into virtually all cell types. They are a major focus of research because of their experimental utility and great promise for therapeutic tissue regeneration. Despite the important role that human ES cells (hESC) could have in biomedical applications, research faces some obstacles. Primarily, spontaneous differentiation can lead to variable levels of differentiated cells within a culture, complicating downstream experiments.

Cell differentiation is often accompanied by the expression of various, possibly lineage-specific, plasma membrane antigens, or markers. For example, B lymphocyte differentiation can be traced, in part, by the expression of CD34, CD45RA, CD19, IgD, and CD27. Since their isolation from the human blastocyst[1], protocols for the consistent *in vitro* maintenance, propagation, and directed differentiation of human stem cells have been tested and continue to be refined[2]. Achieving continuity of lineage homogeneity during manipulations is perhaps the major scientific hurdle to the realization of the potential benefits that human stem cells offer in the clinical setting. This would be greatly assisted with more cell surface markers that could determine the developmental state of hESC and hESC-derived cell populations.

Through a collaborative effort with BresaGen, Inc. (now Novocell, Inc.), Abeome Corporation sought to raise a panel of monoclonal antibodies against hESC plasma membrane antigens (PMAs). By using our proprietary platform technology, DiSH (Direct Selection of Hybridomas), we were able to rapidly select a large number of hESC PMA-positive hybridoma clones derived from mice immunized with BG01v, a laboratory variant of the federally approved BG01 hESC line (NIH registry #BG01). Working together with Millipore Corporation, we then identified two antibodies, HESCA-1 and HESCA-2, which labeled undifferentiated, pluripotent progenitor cells.

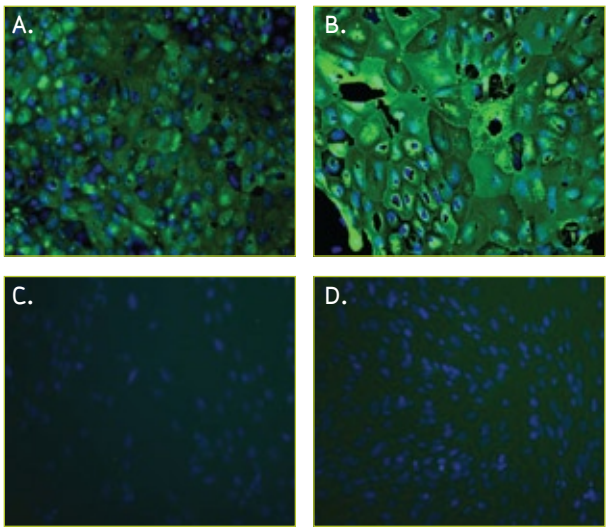

Figure 1. HESCA-1 and HESCA-2 stain hESCs, but not Hs-27 feeders. hESC cells or Hs-27 fibroblasts were cultured in LabTek chamber slides, stained with respective antibody, and counter stained with either FITC- or AlexaFluor® 488-conjugated goat anti-mouse Ig and DAPI. A, BG02 B, BG01v. C, D, Hs-27 cells. A, C, stained with HESCA-1. B, D, stained with HESCA-2.

Methods & Results

Cultured BG01v cells were verified for expression of Oct-4 and SSEA-4[3,4] (not shown), and harvested using enzyme-free cell dissociation buffer (Invitrogen). Cells were washed three times with PBS and adjusted to the appropriate concentration for immunization. Mice were initially immunized *i.p.* with ~10^8 fixed cells in PBS; boosts of ~10^7 cells/per mouse at thirty-day intervals, and ~10^6 cells given three to four days before harvest. Most animals demonstrated serum antibody titers of ≥1:3,000 at the time of harvest.

Abeome's DiSH technology relies on fusing splenocytes with a myeloma (SP2ab) that has been genetically engineered to constitutively express the Igα and Igβ (CD79a and CD79b) components of the B cell antigen receptor (BCR)[5]. Hybridomas generated with SP2ab demonstrate consistent expression of the BCR on their cell surface in addition to maintaining normal secretory capabilities. Thus, Abeome hybridomas can be selected with fluorescent-labeled antigens, and, using a Fluorescent-Activated Cell Sorter (FACS), deposited singly into wells of a 96-well culture plate, thereby eliminating much of the time, labor, and/or cost associated with other hybridoma technologies.

After demonstrating serum antibody titers of ≥1:3,000, mice were sacrificed and splenectomized. Splenocytes were fused with Abeome's SP2ab myeloma cells as described in Harlow and Lane (1988), and cultured in IMDM containing 20% FBS and supplemented with HAT (hypoxanthine, aminopterin, and thymidine; Sigma-Aldrich).

PMA fractions were prepared for labeling by conjugating live, un-fixed, intact BG01v cells with biotin followed by ultrasonic disruption of the cells. Hybridomas were removed from culture with HAT, washed, labeled with biotin-conjugated PMA fractions, and counterstained with streptavidin-conjugated r-phycoerythrin (BD Biosciences) and allophycocyanin-conjugated goat anti-mouse Ig (Invitrogen). Sorting was done using a FACSAria™ Flow Cytometer (BD Biosciences). Cells positive for both Ig-expression and PMA-reactivity were deposited singly into 96-well culture plates containing IMDM supplemented with 20% FBS, hypoxanthine, and thymidine (Sigma-Aldrich).

Verification of positive clones was done by ELISA and immunofluorescence microscopy (IFM) against whole BG01v cells. We were able to identify 158 clones, representing at least 89 identifiable sibling groups. Those that demonstrated clear surface staining by IFM were considered for further evaluation. Two of the clones, 051007-4A5 and 060818-7A6, were of particular interest, and were named HESCA-1 and HESCA-2, respectively. As indicated in Table 1 and Figure 1, both of these clones produced monoclonal antibodies reactive with all hESCs tested (BG01v, BG01, and BG02), but not with feeder cells (murine embryonic fibroblasts (MEF) and Hs-27

	HESCA-1	HESCA-2
Clone	051007-4A5	060818-7A6
Isotype	IgM, κ	IgM, κ
BG01v IFM	+++	+++
BG01 IFM	+++	+++
BG02 IFM	+++	+++
MEF	Negative	Negative
Hs-27	Negative	Negative
mESC	Neg to ± (v. faint)	Negative
Teratomas	Subsets of epithelial cells. Negative for other differentiated cells	Subsets of epithelial cells. Negative for other differentiated cells
Flow Cytometry	(BG02) 92.9%, staining in 4th decade	Not done
Western	~200 kDa	~250 kDa

Table 1. Characteristics of anti-hESC antibodies HESCA-1 & HESCA-2. IFM: immunofluorescence microscopy; BG01v, BG01, and BG02 are hESC cell lines; MEF: murine embryonic fibroblasts; Hs-27: human foreskin fibroblasts; mESC: murine embryonic stem cells.

fibroblasts). HESCA-1 showed some reactivity with mouse ES cells. Both antibodies recognized relatively large proteins in Western blot analysis: ~200 kDa and ~250 kDa, respectively for HESCA-1 and HESCA-2 (Table 1 and not shown).

Since the purpose of this study was to identify potential markers for pluripotent progenitors, teratomas were generated in nude mice using BG01v cells. Teratomas are tumors in which several progenitor differentiation pathways occur. If the novel antibodies could react with differentiated cells, it should be revealed by immunohistochemical analysis of teratomas stained with HESCA-1 and HESCA-2. As demonstrated in Figure 2, neither HESCA-1 nor HESCA-2 showed reactivity with differentiated cells, but did stain epithelioid cells consistent with progenitor cell morphology.

Finally, we analyzed BG02 hESCs for reactivity with HESCA-1 and Tra-1-81 by flow cytometry. Tra-1-81 is generally considered a reliable marker for pluripotency[3,4]. Similar to Tra-1-81, HESCA-1 stained >90% of BG02 cells (Figure 3), suggesting that Tra-1-81 and HESCA-1 are co-expressed on the same population of cells.

Conclusion

The data suggest that HESCA-1 and HESCA-2 identify hESC lines as well as populations of cells consistent with undifferentiated pluripotent progenitor cells. These novel antibodies potentially represent new tools for research on stem cells, particularly for the identification of undifferentiated cells.

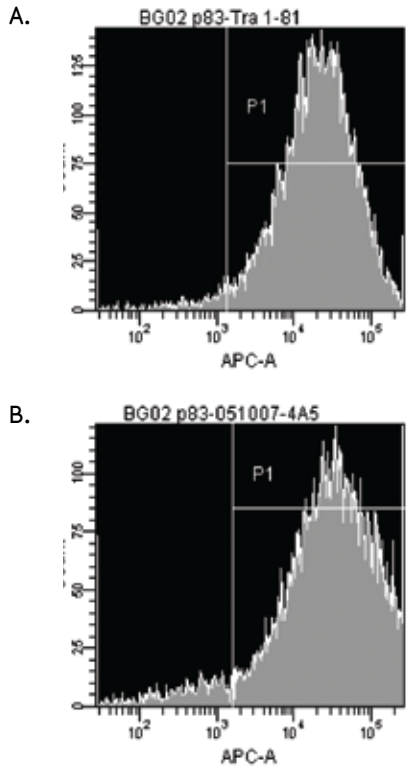

Figure 3. Flow cytometry analysis of BG02 cells stained with A) Tra-1-81 (P1 = 96.6%), and B) HESCA-1 (051007-4A5, P1 = 92.9%).

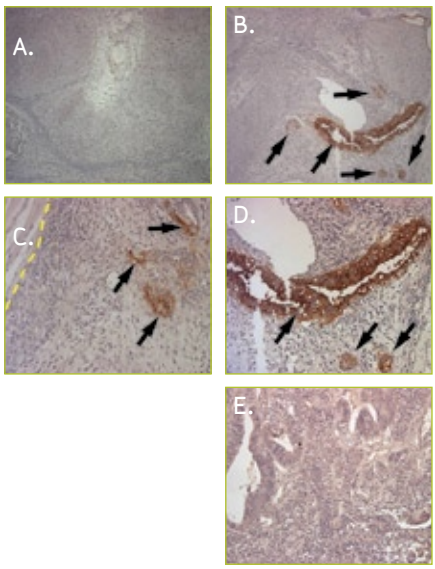

Figure 2. BG02-derived teratomas stained with HESCA-1 (A – D) and HESCA-2 (E). A, B, 10X magnification; C– E, 20X magnification. Arrows indicate positive-stained cells. Dotted line indicates border between teratoma and host tissue.

References

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Through our collaboration with Abeome Corporation, Millipore is proud to be the exclusive provider of these innovative antibodies, HESCA-1 and HESCA-2.

Millipore Products

Description	Catalogue No.
Anti-HESCA-1, clone 051007-4A5, 100 µg	MAB4407
Anti-HESCA-2, clone 060818-7A6, 100 µg	MAB4406
Anti-Stage-Specific Embryonic Antigen-4 [SSEA-4], clone MC-813-70	MAB4304
Anti-Stage-Specific Embryonic Antigen-3 [SSEA-3], clone MC-631	MAB4303
Anti-TRA-1-60, clone TRA-1-60	MAB4360
Anti-TRA-1-81, clone TRA-1-81	MAB4381
Anti-TRA-1-85, blood group antigen Ok(a), clone TRA-1-85	MAB4385
Anti-TRA-2-54, clone TRA-2-54/2J	MAB4354
Anti-ShSCP-5, clone 8H9.3	MAB4408
Anti-Oct-4, clone 10H11.2	MAB4401
Anti-Sox-2, clone 6F1.2	MAB4343
Anti-UTF-1, clone 5G10.2	MAB4337
Anti-Pramel-4	AB4304
Anti-Pramel-5	AB4305
Anti-Nanog, N-terminus	AB5731
Anti-Nanog	AB9220
Anti-Nucleostemin	AB5689

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