APPLICATION NOTE

Anti-hESC mAb Hesca-2 Binds to a Glycan Epitope Commonly Found on Carcinomas

DiSH™ Kit (ENZ-71001-0001)

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Hesca-2, a monoclonal antibody (mAb) IgM raised to the human embryonic stem cell (hESC) line BG-01v, binds with high affinity (nM) to the disaccharide epitope [Gal1- 3GlcNAc] on a glycan microarray. This glycan epitope, found on glycoproteins, glycolipids and free oligosaccharides in milk, is recognized by a number of human galectins particularly galectin-7. It was expressed on pluripotent progenitor hESC cells in culture, but not in various differentiated cells derived from hESC-based on immunofluorescence microscopy (IFM). Interestingly, this glycan epitope is also associated with carcinomas including pancreatic, stomach, colorectal and ovarian. Hesca-2 cross-reacts in IFM studies with several human ovarian cancer cell lines and is cytotoxic to them based on the release of cytosolic enzyme lactatedehydrogenase (LDH) into the media. Hesca-2 also has an negligible cytotoxic activity at low concentrations on hESCs in culture and a more dramatic and different effect on the cells at higher concentrations that may be due to cytoprotection or proliferation. The mechanism of this activity clearly requires multimeric binding in that Fab fragments do not show a comparable response. Future studies should explore the precise mechanism(s) involved in these observed disparate activities; in particular, assessing what affect the soluble glycan ligands (recognized by Hesca-2) have on the proliferation, adhesion, etc. of hESCs. There is an intriguing possibility that the glycan ligands recognized by Hesca-2 and galectin-7 (or another galectin) could be involved in processes critical for both stem cell and cancer cell survival or proliferation.

PURPOSE

1. To define and produce a number of monoclonal antibodies (mAbs) against human embryonic stem cells (hESCs).

2. To identify and initiate characterization of novel monoclonal antibodies (mAbs) specific to hESC plasma membrane antigens

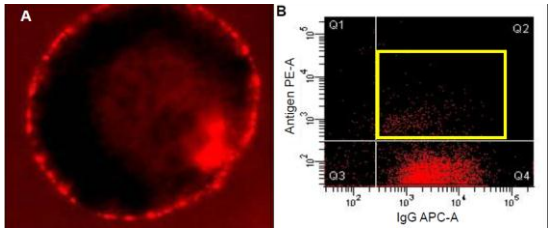
METHOD

DiSH™ Technology

~10^8 hES cells
(Followed at 28-day intervals with boosts of ~10^7 cells)

Splenocytes are fused with Abeome's myeloma cell line, Sp2ab

Cells reactive for both hES cell surface proteins and Ig surface expression are selected by FACS and singly deposited into wells of 96-well plate



Cells are sorted based on surface fluorescence by staining for both specific antigen and surface Ig. (A) Control hybridoma stained with APC-conjugated goat anti-mouse Ig. (B) Cells (boxed) positive for antigen (y axis) and for surface Ig (x axis) were deposited singly into wells of 96-well culture plates. Thus, we are able to ensure monoclonality by single cell sorting.

Screening for Novel mAbs:

- **Antigen Reactivity**
  - ELISA
  - Immunofluorescence Microscopy (IFM)
  - Immunohistochemistry (IHC)
- **Functional Activity**
  - Cell Death (LDH Activity)
  - Impedance
- **Target Identification**
  - Western Immunoblot
  - Glycan Array Analysis

RESULTS

IFM was used to discern the staining pattern of Hesca-2 on hESCs, mESCs, and the various feeder cells used in culturing embryonic stem cells.

Immunofluorescent staining of Hesca-2

BG-02 human embryonic stem cells (hESCs)

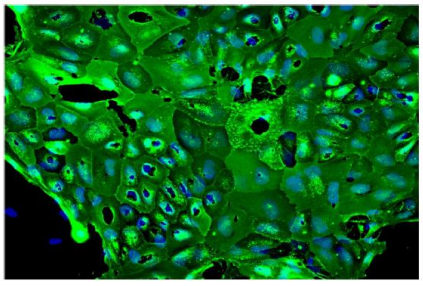


Figure 1. Immunofluorescent stainging by Hesca-2 on BG-02 hESCs culture in LabTek chamber slides. Slides were stained with Hesca-2 and counterstained with goat anti-mouse Ig conjugated to FITC (green). Nuclear staining appears in blue (DAPI); 20X magnification.

Human epithelial ovarian cancer lines OVCAR3 and SKOV3 were also assessed with the Hesca-2 antibody. There was weak staining on these cells.

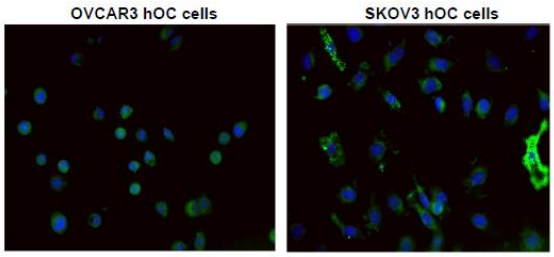


Figure 2. Immunofluorescent stainging by Hesca-2 on hOCs OVCAR-3 and SKOV3 cultured in LabTek chamber slides. Slides were stained with Hesca-2 and counterstained with goat anti-mouse Ig conjugated to FITC (green). Nuclear staining appears in blue (DAPI); 40X magnification.

The immunohistochemical staining of patient tumor tissue on TMAs was examined with Hesca-2. Staining was observed on a number of common tumor types as shown in Fig. 3.

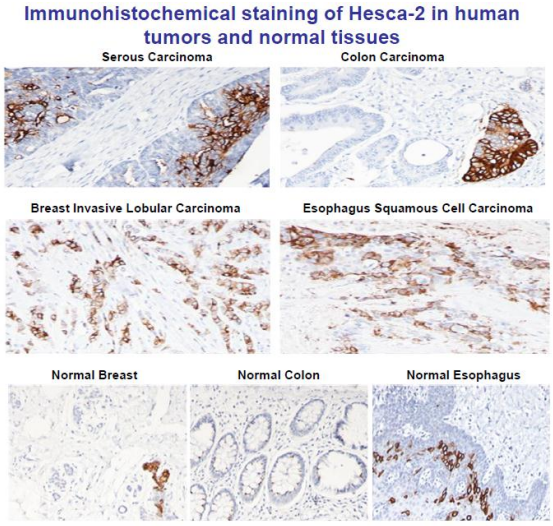


Figure 3. Patient tissues microarrays (TMAs) prepared at the Fox Chase Cancer Center Biosample Repository (Philadelphia, PA) were stained with Hesca-2 (1:500 dilution) and counterstained with hematoxylin (Gil II SurgiPath).

The Hesca-2 antibody displayed cytotoxic properties when incubated with select cell types at a broad range of concentrations (Fig. 4). Higher doses proved to be less potent. Cell specific effects were also observed.

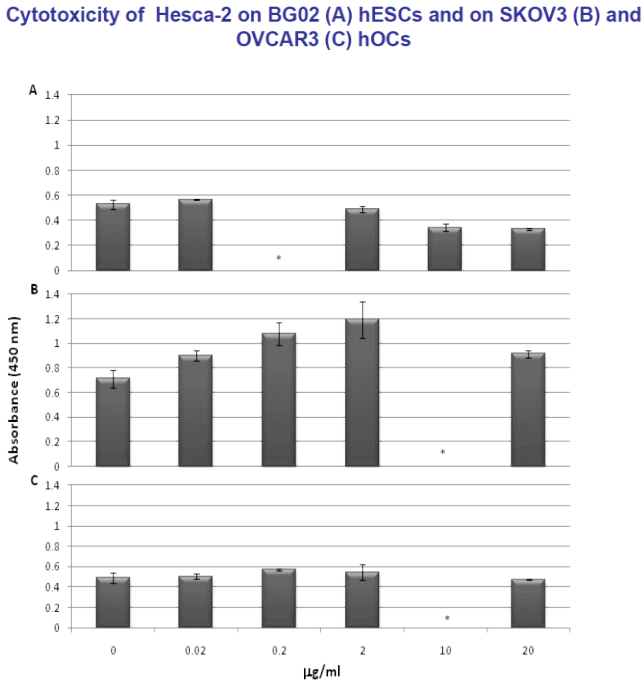


Figure 4. The effect of different concentrations of Hesca-2 on LDH enzyme release on (A) hESCs, (B) SKOV3 and (C) OVCAR3 cells in culture. Tests were done in at least triplicate and the results averaged. Standard deviations were computed and are shown as error bars.

Cell growth was also monitored using the xCELLigence system, which measures electrical impedance. The cell index (proportional to cell density, viability, and morphology=adhesion) monitored in real time over a 72-h period corroborates the LDH data.

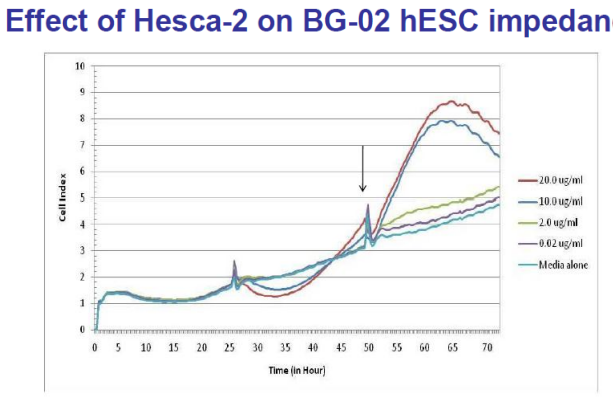


Figure 5. Cell growth/death measure with impedance analysis in real-time of BG02 cells treated with various concentrations of Hesca-2. Antibody addition begins at 24 hr requiring the plate to be disconnected from the analyzer. (After an additional 24 hr (arrow) and 48 hr (not shown) aliquots are taken for LDH assays). Tests performed in quadruplicate and the results averaged.

Western blot analysis (not shown) on the BG-01v cell lysate probed with Hesca-2 revealed a major species migrating at an apparent molecular weight of 250 kDa with a diffuse pattern of staining suggestive of a mucin.

To determine if the antigen recognized by Hesca-2 was a glycan, the antibody was screening using a glycan array. The glycan array contained a diverse set of 204 carbohydrates and glycoproteins. This screen demonstrated specific binding to a number of oligosaccharides containing the type 1 chain as seen in Fig. 6, including blood group H1 (BG-H1), lacto-N-tetraose (LNT), lacto-N-hexaose (LNH), and LeC (also referred to as the type 1 precursor) presented as neoglycoproteins of varying ligand densities.

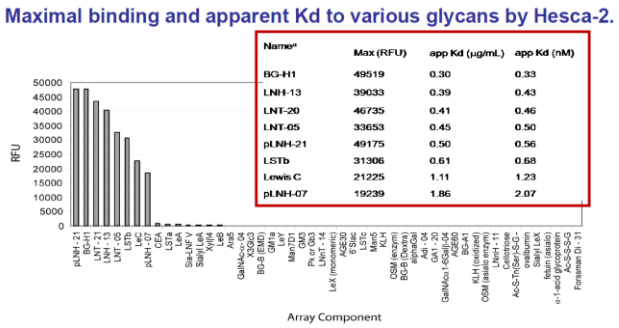
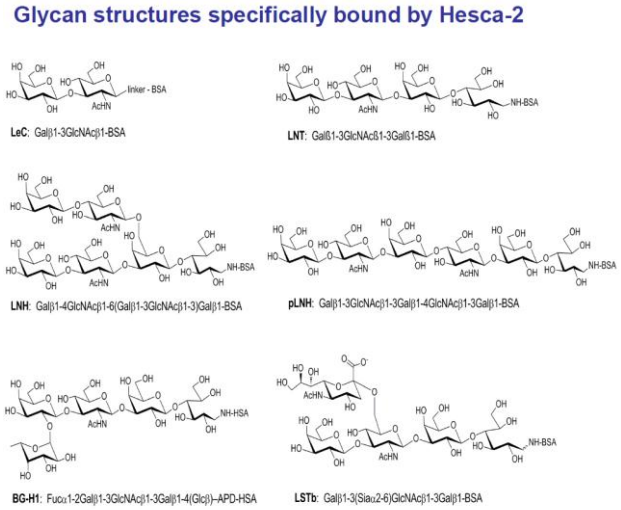


Figure 6. Glycan array for screening Hesca-2.

On the basis of the binding profile, the minimum glycan epitope is the disaccharide, Galb1-3GlcNAc.



CONCLUSIONS

Using DiSH™ Technology, a novel antibody was rapidly isolated and characterized for reactivity and functionality. The antibody, Hesca-2, binds with high affinity to the disaccharide epitope, Galb1-3GlcNAc, which is present on both stem cells and some cancer cells. The glycan ligand recognized by Hesca-2 could be involved in processes critical for both stem cell and cancer cell survival or proliferation.

REFERENCES

1. P.W. Price, *et al.* Engineered cell surface expression of membrane immunoglobulin as a means to identify monoclonal antibody-secreting hybridomas. *J. Immunol. Meth.* 343 (2009) 28-41

2. M.G. Shoreihah, *et al.* Anti-Human Embryonic Stem Cell Monoclonal Antibody Hesca-2 Binds to a Glycan Epitope Commonly Found on Carcinomas. *Stem Cells and Development* 20 (2011) 515

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