

UNITED STATES
SECURITIES AND EXCHANGE COMMISSION

Washington, D.C. 20549

FORM 8-K

CURRENT REPORT

Pursuant to Section 13 or 15(d) of the Securities Exchange Act of 1934

Date of Report (date of earliest event reported): **April 21, 2015**

BioTime, Inc.

(Exact name of registrant as specified in its charter)

California
(State or other jurisdiction of incorporation)

1-12830
(Commission File Number)

94-3127919
(IRS Employer Identification No.)

1301 Harbor Bay Parkway
Alameda, California 94502
(Address of principal executive offices)

(510) 521-3390
(Registrant's telephone number, including area code)

Check the appropriate box below if the Form 8-K filing is intended to simultaneously satisfy the filing obligation of the registrant under any of the following provisions:

- Written communications pursuant to Rule 425 under the Securities Act (17 CFR 230.425)
- Soliciting material pursuant to Rule 14a-12 under the Exchange Act (17 CFR 240.14a-12)
- Pre-commencement communications pursuant to Rule 14d-2(b) under the Exchange Act (17 CFR 240.14d-2(b))
- Pre-commencement communications pursuant to Rule 13e-4(c) under the Exchange Act (17 CFR 240.13e-4(c))

Forward-Looking Statements

Any statements that are not historical fact (including, but not limited to statements that contain words such as “may,” “will,” “believes,” “plans,” “intends,” “anticipates,” “expects,” “estimates”) should also be considered to be forward-looking statements. Additional factors that could cause actual results to differ materially from the results anticipated in these forward-looking statements are contained in BioTime’s periodic reports filed with the Securities and Exchange Commission (“SEC”) under the heading “Risk Factors” and other filings that BioTime may make with the SEC. Undue reliance should not be placed on these forward-looking statements which speak only as of the date they are made, and the facts and assumptions underlying these statements may change. Except as required by law, BioTime disclaims any intent or obligation to update these forward-looking statements.

This Report and Exhibit 99.1 shall be deemed “furnished” and not “filed” under the Securities Exchange Act of 1934, as amended.

Section 7 - Regulation FD

Item 7.01 - Regulation FD Disclosure

A poster, including an abstract of the results of work by BioTime scientists with human embryonic stem cell-derived clonal brown adipocyte progenitors, presented by BioTime at the Keystone Symposia on Molecular and Cellular Biology on Sunday, April 19, 2015, is now available on BioTime’s website at www.recyte.com and is furnished with this report as Exhibit 99.1.

Section 9 - Financial Statements and Exhibits

Item 9.01 - Financial Statements and Exhibits.

<u>Exhibit Number</u>	<u>Description</u>
99.1	Poster presentation

SIGNATURES

Pursuant to the requirements of the Securities Exchange Act of 1934, the registrant has duly caused this report to be signed on its behalf by the undersigned hereunto duly authorized.

BIOTIME, INC.

Date: April 21, 2015

By: s/Michael D. West
Chief Executive Officer

HUMAN EMBRYONIC STEM CELL-DERIVED CLONAL BROWN ADIPOCYTE PROGENITORS

Hal Sternberg, David Larocca, Michael D. West
BioTime, Inc., Alameda, CA, USA



ABSTRACT

Emerging strategies for the treatment of metabolic disorders via the transplantation of brown adipose tissue (BAT) cells will require a robust and scalable source of highly-defined cells as well as a matrix to promote reliable engraftment. Toward this end, we used a novel modality of screening scalable human embryonic stem (hES) cell-derived clonal progenitor lines for BAT differentiation in HyStem®-C hydrogel, a matrix currently in a pivotal human clinical trial for lipotransfer. We screened >100 diverse clonal embryonic progenitor cell lines (PureStem™, ESIBIO) from diverse embryological anlagen and site-specific homeobox gene expression by differentiating the lines in HyStem-C supplemented with combinations of adipogenic inducers including: the PPARγ agonist rosiglitazone, BMP4, T3, and the β3-adrenergic agonist CL316243. The clonal progenitor cell lines commonly showed adipogenic potential as evidenced by the expression of *FABP4* and *CD36*; however, three relatively rare families of clones displayed the capacity to also express either *lipasin* (*C19orf80*) and *adiponectin* (*ADIPOQ*), *UCP1*, or a combination thereof. The clonal progenitor line designated E3 representing Class I, displayed strong expression of *lipasin* and *ADIPOQ* but very low levels of *UCP1*. The line C4EL5.1 representing Class II showed induction of *UCP1*, but little to no expression of *lipasin* and *ADIPOQ* following differentiation. Significantly, the line NP110SM representing Class III, expressed the site-specific *HOX* gene expression marker *HOXA5* consistent with a thoracic location. The Class III lines induced higher levels of *UCP1* transcript than Class I or II cells or fetal BAT-derived cells, as well as relatively high levels of *lipasin* and *ADIPOQ* expression. Clonally hES cell-derived progenitors are capable of industrial level scale-up and differentiation to BAT-like cells when differentiated in HyStem matrix known to be safe in humans. Further characterization of these lines in preclinical studies may illumine their potential for therapeutic application in metabolic disorders such as obesity, diabetes, hypertension, and coronary disease.

Basal Adipogenic Medium: DMEM high glucose (CellGro Cat. No. 15-013-CV), Pyruvate, 1mM (Gibco Cat. 11360), Pen:Strep 100U/ml:100ug/ml (Gibco Cat. No. 504284), Glutamax 2mM (Gibco Cat. No. 35050), Dexamethasone 0.1uM (Sigma, St. Louis, MO, Cat. No.D1756-100), L-Proline 0.35mM (Sigma Cat. No. D49752), 2-phospho-L-Ascorbic Acid 0.17mM (Sigma, Cat. No. 49792, Fluka), ITS Premix (BD, Franklin Lakes, NJ, sterile Cat. No. 47743-628) final concentration 6.25ug/ml insulin, 6.25ug/ml transferrin, 6.25ng/ml selenious acid, serum albumin 1.25mg/ml, 5.35 ug/ml linoleic acid.

Brown Adipocyte Differentiation: Cells are suspended in HyStem solution at 20-25 x10⁶ cells/ml (according to manufactures directions). Multiple 25ul aliquots are placed in either wells of a 6 well plate or 60mm dishes. Following gelation (in 30-40 minutes) differentiation medium is added consisting of "Basal Adipogenic medium" with combinations of the supplements BMP4 (Humanzyme, Chicago IL, Cat# HZ-1078) 10-50ng/ml, rosiglitazone 1-5uM (Cayman Chem, Ann Arbor MI, Cat# 71740) and T3 2nM (Sigma Cat# T6397). The cells are fed M,W, and Friday. On day 14 or day 21, 4 hours before harvest of RNA, or fixation, CL316243 10uM (Torcis Cat# 1499) is added to the differentiation cocktail.

Gene Expression Analysis: Total RNA was extracted directly from cells using Qiagen RNeasy mini kits according to the manufacturer's instructions. RNA concentrations were obtained using a Beckman DU530 or Nanodrop spectrophotometer and RNA integrity was determined by denaturing agarose gel electrophoresis or by an Agilent 2100 bioanalyzer. Whole-genome expression analysis was performed using Illumina Human HT-12 v4 BeadArrays, and RNA expression levels for certain genes were verified by qRT-PCR. For the Illumina BeadArrays, total RNA was linearly amplified and biotin-labeled using Illumina TotalPrep kits (Life Technologies, Temecula, CA, USA). The cRNA quality was controlled using an Agilent 2100 Bioanalyzer, and was hybridized to Illumina BeadChips, processed, and read by a BeadStation array reader according to the manufacturer's instructions (Illumina, San Diego, CA, USA). Values under 100 relative fluorescence units (RFUs) were considered as nonspecific background signal.

RESULTS

Screening of Diverse hES Cell-Derived Clonal Progenitor Cell Lines for Adipocyte Progenitor Cell (APC) Fate Potential:

FIGURE 1: Expression of the Adipocyte Marker *FABP4* in Human Fetal BAT-Derived Preadipocytes and Diverse hES Cell-Derived Clonal Progenitor Cell Lines Differentiated in HyStem/BMP4 as Determined by Illumina Microarray Analysis

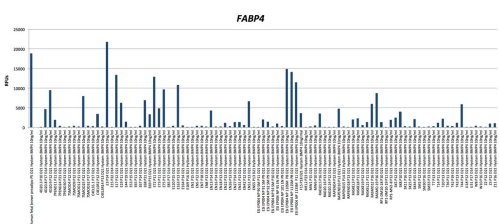


FIGURE 2: Type I-III APCs Show Unique Patterns of *Lipasin*, *ADIPOQ*, and *UCP1* Expression

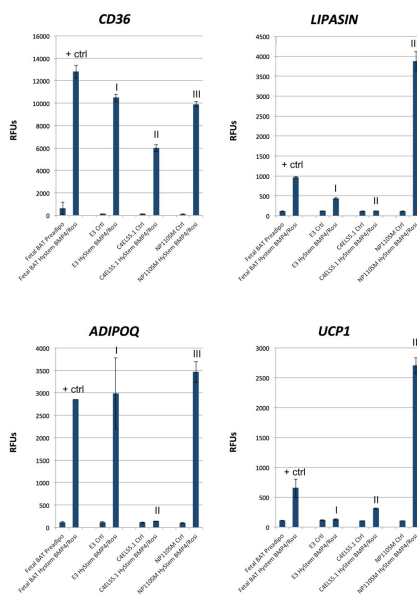


FIGURE 3: Types I, II, and III APCs show Evidence of Diverse Embryological Origins with Site-Specific Markers

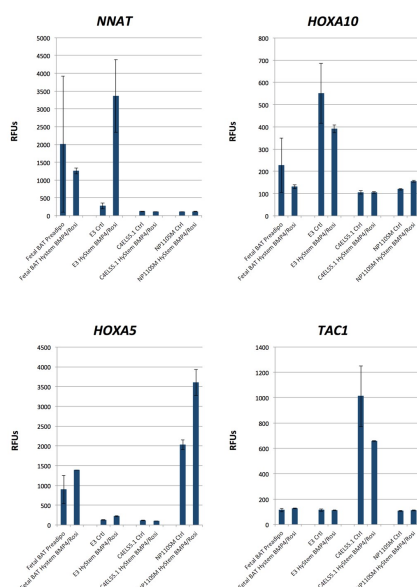


FIGURE 4: Optimizing Differentiation Conditions in the Cell Line NP110SM

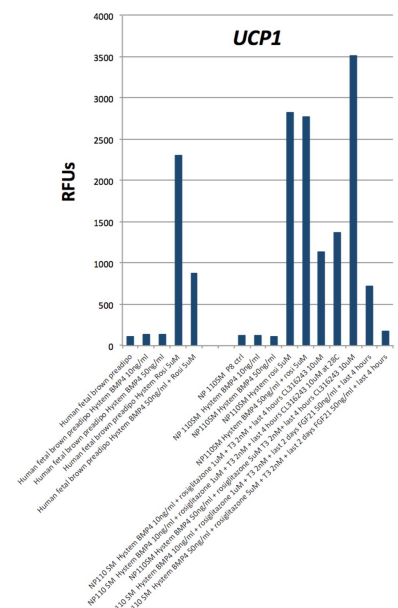
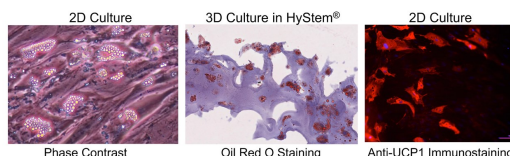


FIGURE 5: Lipid accumulation and UCP1 in differentiated NP110SM



SUMMARY

hES Gene	Fetal BAT (+Ctrl)	E3	C4EL5.1	NP110SM
<i>FABP4</i>	+	+	+	+
<i>Lipasin</i>	+	+	-	+
<i>ADIPOQ</i>	+	+	-	+
<i>UCP1</i>	+++	-/+	++	+++
<i>ELOVL3</i>	+	+	+	-
<i>HOXA5</i>	+	-	-	+

CONCLUSIONS

- A wide diversity of clonal adipocyte progenitor cells can be isolated from hPS cells
- The 3 lines studied overexpress adipocyte marker *FABP4* upon growth in differentiation conditions. NP110SM has highest level induction.
- Type I (i.e. E3 cell line) expresses *lipasin* and *ADIPOQ* but very low or undetectable levels of *UCP1* upon differentiation.
- Type II (i.e. C4EL5.1) express *UCP1* but not *lipasin* or *ADIPOQ* upon differentiation.
- Type III (i.e. NP110SM) expresses *UCP1*, *lipasin*, and *ADIPOQ* upon differentiation at levels exceeding fetal brown fat cells.
- Lipid droplets typical of BAT are seen in Oil Red-O stained differentiated cells in 2D monolayer culture and in 3D HyStem culture.
- Clonal derivation of BAT progenitors and growth in 3D HyStem-C cell matrix is a promising platform for obtaining a scalable source of highly defined BAT cells combined with an injectable matrix for transplantation *in vivo*.