pharmaceutical companies to apply the three "Rs" principle to the potency testing of botulinum neurotoxins: Reduce, Refine, Replace. D. Straughan, *Progress in Applying the Three Rs to the Potency Testing of Botulinum Toxin Type A*, Altern. Lab. Anim. 34(3): 305-313 (2006). In recent years, several steps have been already taken to reduce and refine the mouse LD_{50} bioassay in order to standardize the protocol and produce more consistent data using fewer animals per assay.

[05] Thus, a simple, reliable, validated and governmental agency acceptable botulinum toxin activity assay that can evaluate the integrity of all the steps necessary in botulinum toxin uptake would be of significant value because such a non-animal based assay would alleviate the need for animal testing and all the disadvantages, costs and ethical concerns associated with this type of animal-based assay. Companion patent application Ester Fernandez-Salas, et al., *Immuno-Based Botulinum Toxin Serotype A Activity Assays*, U.S. Patent Application Serial No: 12/403,531, provides novel compositions, cells, and methods for assaying the activity of a BoNT/A useful for various industries, such as, *e.g.*, the pharmaceutical and food industries. Such compositions, cells, and methods do not use live animals or tissues taken from live animals, but can evaluate all the steps necessary for neurotoxin action, namely, binding and cellular uptake of toxin, translocation into the cell cytosol, and protease activity.

[06] One of the necessary components for the methods disclosed in U.S. Patent Application Serial No: 12/403,531 is the use of cells from an established cell line that are susceptible to low levels of BoNT/A intoxication because the amount of BoNT/A contained within a commercially-available pharmaceutical composition is very low. For example, approximately 4-5 ng of a BoNT/A complex is contained in the pharmaceutical composition sold as BOTOX®. Thus, there is a need to identify and create established cell lines comprising cells that are susceptible to BoNT/A intoxication when only very low levels of the neurotoxin is present in the sample. The present specification provides novel cells and cell compositions. that are susceptible to BoNT/A intoxication when only very low amounts of BoNT/A is present and thus allow assaying of commercially-available pharmaceutical composition comprising BoNT/A.

SUMMARY

[06a] In an aspect, there is provided an established single cell derived clonal cell line from SiMa cell line DSMZ ACC 164 comprising cells susceptible to intoxication by botulinum toxin type A (BoNT/A),

wherein the cells exhibit at least a 1.5-fold increase in mRNA expression levels after differentiation in the genes ADAMTS9, ATAD2, CDC45L, CNTN1, Cyclin A, Cyclin E, E2F1, E2F2, E2F7, FGFR2, MYBL1, MYBL2, NDC80, NDN, ORC1L, PLS3, RAD54L, RBL1, RRM2, S1PR3, SPC24, SPC25, TFDP1, TFPI2, TK1, TTK, TYMS, TYK and ZWINT relative to the mRNA expression levels of each of these genes in cells from a 2D6 derived from a parental SiMa cell line DSMZ ACC 164; and,

wherein the cells exhibit at least a 1.5-fold decrease in mRNA expression levels after differentiation in the genes CDKN2A and CNTN2 as compared to the mRNA expression levels of each of these genes in cells from the 2D6 cell line; and,

wherein the clonal cell line comprises cells susceptible to BoNT/A intoxication by about 100 pM or less of a BoNT/A.

BRIEF DESCRIPTION OF THE DRAWINGS

[07] **FIG. 1** shows a schematic of the current paradigm of neurotransmitter release and Clostridial toxin intoxication in a central and peripheral neuron. FIG. 1A shows a schematic for the neurotransmitter release mechanism of a central and peripheral neuron. The release process can be described as comprising two steps: 1) vesicle docking, where the vesicle-bound SNARE protein of a vesicle containing neurotransmitter molecules associates with the membrane-bound SNARE proteins located at the plasma membrane; and 2) neurotransmitter release, where the vesicle fuses with the plasma membrane and the neurotransmitter molecules are exocytosed. FIG. 1B shows a schematic of the intoxication mechanism for tetanus and botulinum toxin activity in a central and peripheral neuron. This intoxication process can be described as comprising four steps: 1) receptor binding, where Clostridial toxin binding to a Clostridial receptor complex and initiates the intoxication process; 2) complex internalization, where after toxin binding, a vesicle containing a toxin/receptor system complex is endocytosed into the cell; 3) light chain

What is claimed:

1. An established clonal cell line comprising cells susceptible to intoxication by BoNT/A,

   wherein the cells exhibits at least a 1.5-fold difference in gene expression levels of three or more genes as compared to the expression levels of these genes in cells from the parental SiMa cell line DSMZ ACC 164; and

   wherein the gene expression levels are selected from th group ADAMTS9, ASCL1, BASP1, DOK5, DPYD, GNB4, GNG11, GTSF1, MAOA, MINA, MSN, PEG3, PLK2, PRSS12, RNF182, SLC44A5, SPARC, TFPI2, and TPTE; and

   wherein the the clonal cell line comprises cells susceptible to BoNT/A intoxication by about 100 pM or less of a BoNT/A.

2. The established clonal cell line of Claim 1, wherein the the cells exhibit at least a 2.0-fold difference in gene expression levels of one or more genes as compared to the expression levels of these genes in cells from the parental SiMa cell line DSMZ ACC 164, at least a 3.0-fold difference in gene expression levels of one or more genes as compared to the expression levels of these genes in cells from the parental SiMa cell line DSMZ ACC 164, or at least a 4.0-fold difference in gene expression levels of one or more genes as compared to the expression levels of these genes in cells from the parental SiMa cell line DSMZ ACC 164.

3. The established clonal cell line of Claim 1, wherein the gene expression levels are measured before differentiation of the cell or after differentiation of the cell.

4. The established clonal cell line of Claim 1, wherein the cell from an established clonal cell line exhibits about 100 pM or less of a BoNT/A for at least 5 or more cell passages.

What is claimed:

1. An established single cell derived clonal cell line from SiMa cell line DSMZ ACC 164 comprising cells susceptible to intoxication by botulinum toxin type A (BoNT/A),

   wherein the cells exhibit at least a 1.5-fold increase in mRNA expression levels after differentiation in the genes ADAMTS9, ATAD2, CDC45L, CNTN1, Cyclin A, Cyclin E, E2F1, E2F2, E2F7, FGFR2, MYBL1, MYBL2, NDC80, NDN, ORC1L, PLS3, RAD54L, RBL1, RRM2, S1PR3, SPC24, SPC25, TFDP1, TFPI2, TK1, TTK, TYMS, TYK and ZWINT relative to the mRNA expression levels of each of these genes in cells from a 2D6 derived from a parental SiMa cell line DSMZ ACC 164; and,

   wherein the cells exhibit at least a 1.5-fold decrease in mRNA expression levels after differentiation in the genes CDKN2A and CNTN2 as compared to the mRNA expression levels of each of these genes in cells from the 2D6 cell line; and,

   wherein the clonal cell line comprises cells susceptible to BoNT/A intoxication by about 100 pM or less of a BoNT/A.

2. The established clonal cell line of claim 1, wherein the cells exhibit at least a 2.0-fold difference in gene expression levels of at least one gene as compared to the expression levels of the gene in cells from the parental SiMa cell line DSMZ ACC 164.

3. The established clonal cell line of claim 1, wherein the cells exhibit at least a 3.0-fold difference in gene expression levels of at least one gene as compared to the expression levels of the gene in cells from the parental SiMa cell line DSMZ ACC 164.

4. The established clonal cell line of claim 1, wherein the cells exhibit at least a 4.0-fold difference in gene expression levels of at least one gene as compared to the expression levels of the gene in cells from the parental SiMa cell line DSMZ ACC 164.

5. The established clonal cell line of claim 1, wherein the cell from an established clonal cell line exhibits about 100 pM or less of a BoNT/A for at least 5 or more cell passages.