NEURODEGENERATIVE DISEASE THERAPIES UTILIZING THE SKIN-BRAIN AXIS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 62/869,788, filed July 2, 2019, which is hereby incorporated herein by reference in its entirety.

BACKGROUND

[0002] Amyloidosis refers to a group of pathologies characterized by the toxic misfolding of proteins into β-pleated sheet fibrils leading to either a systemic or local aggregation and deposition in specific organs or tissues (Merlini, G. & Bellotti, V. N. Engl. J. Med. 2003 349:583–596). Numerous proteins and peptides forming amyloid deposits are associated with multiple human diseases targeting several organs (Chiti, F. & Dobson, CM. Annu. Rev. Biochem. 2017 86:27–68). For instance, neurodegenerative diseases such as Alzheimer's Disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD), are all considered local amyloidosis in which characteristic pathogenic amyloid proteins aggregate in the brain (Tillement, J.-P., et al. Pharmacology 2010 85:1–17).

[0003] Alzheimer's Disease (AD) is a neurodegenerative disease characterized by memory loss and a progressive detriment of cognitive and executive functions and constituting the most common form of dementia, currently affecting more than 50 million people worldwide (Alzheimers. Dement. 2020 doi:10.1002/alz.12068). Accumulating evidence establishes a causal relationship between neuronal cell loss and the accumulation of neuropathological lesions formed by amyloid-beta (Aβ) plaques and phosphorylated Tau tangles inside AD-affected brains (Small, SA. & Duff, K. Neuron 2008 60:534–542).

SUMMARY

[0004] As disclosed herein, the skin can dispatch signals to the brain in the form of exosomes, and exosomes derived from the skin of Alzheimer's Disease (AD) subjects contain neurotoxic cargo that could potentially be impacting the progression of this disease. This is a paradigm-shifting concept in AD, referred to herein as a "skin-brain axis."

[0005] In some embodiments, exosomes derived from the skin can be cumulatively carrying neurotoxic cargo to the brain and contributing to the onset and/or progression of neurodegenerative diseases such as Alzheimer's Disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) (i.e., skin-brain axis).

[0006] Therefore, disclosed herein is a method for diagnosing a neurodegenerative disease in a subject that involves isolating exosomes from the subject and assaying the exosomes for the presence of one or more biomarkers. In some embodiments, the method involves assaying the exosomes for the regulation of one or more pathways related to neurodegenerative disease. For example, the method can involve assaying for dysregulation of any pathway associated with a brain disorder, disease, or injury.

[0007] Moreover, as disclosed herein exosome engineering approaches can be used as a therapeutic strategy for the brain, using the skin as a "window" to the brain. In some embodiments, the disclosed compositions and methods can be used to treat any injury, disease, or disorder of the brain by delivering a therapeutic gene or cargo. In some embodiments, the brain disease is a neurodegenerative disease such as Alzheimer's Disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD). In some embodiments, the brain disease is a brain cancer, including primary and secondary brain cancers. In some embodiments, the brain disease or injury involves a stroke or ischemia. In some embodiments, the brain injury is a traumatic brain injury.

[0008] Therefore, disclosed herein are methods of treating a subject that involves engineering the skin of the subject to produce therapeutic exosomes.

[0009] Also disclosed are methods of collecting skin-produced exosomes and loading them with therapeutic cargo, such as those described herein that can treat one or more diseases, disorders, or injuries of the brain.

[0010] Also disclosed herein is a method to reduce exosomal release from the skin to reducing trafficking to the brain. For example, in some embodiments, neutral sphingomyelinase inhibitor GW4869 can be applied topically and/or via intradermal injection to reduce skin-exosome release.

[0011] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

[0012] FIG. 1 shows proposed skin-brain axis and its role in delivering neurotoxic cargo to the brain via exosomes, contributing to the development and/or progression of AD.

[0013] FIG. 2A shows TNT used to deliver CD63-GFP plasmids to the skin of healthy mice to label the exosomes. Control skin was TNT-treated with mock/empty plasmids. FIG. 2B shows GFP signal detected in the brain 24 hours post-TNT on the skin (absent in

control), suggesting that skin-derived exosomes appeared to be able to enter circulation and lodge in the brain.

[0014] FIG. 3A shows qRT-PCR data of the relative expression of a mutated human version of amyloid precursor protein (hAPP) mRNA only seen in AD vs. healthy mice. FIG. 3B shows immunoblot analysis of amyloid-beta protein in skin-derived exosomes from healthy and AD mice confirming the presence only under AD.

[0015] FIGs. 4A to 4C show differential expression and IPA ontology analysis on RNA content of skin EVs. Volcano plots presenting differential gene content in EVs (Top) and Canonical pathways enriched by differential gene content (Bottom) of 3xTg-AD compared with B6129SF2/J at 23 weeks (FIG. 4A); B6129SF2/J and 3xTg-AD at 23 weeks compared with 10 weeks (FIG. 4B); 3xTg-AD 23 weeks compared with B6129SF2/J 10 weeks, and 3xTg-AD 10 weeks compared with B6129SF2/J 23 weeks (FIG. 4C). For canonical pathway analysis, blue bars represent pathway downregulation (z–score < 0), white bars represent (z-score = 0), and gray bars represent no pattern of regulation.

[0016] FIGs. 5A and 5B shows skin-derived EVs from 3xTg-AD mice contain mRNA of transgenic genes for human APP and MAPT. Absolute qPCR data of skin-derived exosomes from B6129SF2/J and 3xTg-AD mice indicating the presence of transgenic human APP (FIG. 5A) and human MAPT (FIG. 5B) in 3xTg-AD EVs.

[0017] FIGs. 6A and 6B show qPCR data of primary neuron cultures exposed to 3xTg-AD and B6129SF2/J skin-derived EVs for 24 hours measuring expression of transgenic hAPP (FIG. 6A) and hMAPT (FIG. 6B). mRNA of transgenic hAPP and hMAPT was found in murine primary embryonic neurons exposed to skin-derived EVs from 3xTg-AD mice and absent and controls.

[0018] FIGs. 7A to 7C show 60x deconvolution (FIG. 7A) and confocal images (FIG. 7B) of skin-derived EVs from 3xTg-AD mice that were fluorescently labeled using PKH26 Red Fluorescent Cell Linker Kit (Sigma). The fluorescently labeled EVs were exposed to murine primary neuron cultures, followed by ICC with neuron specific TUJ1 and stained with DAPI. FIG. 7C shows resulting quantification of immunofluorescence data shows labeled EVs in and around $TUJ1^{+}$ cells at significantly greater levels than $TUJ1^{-}$.

[0019] FIGs. 8A and 8B show Live/Dead cell viability assay of murine primary embryonic neuron cultures exposed to 3xTg-AD/B6129SF2/J skin-derived EVs. FIG. 8A shows 20X fluorescent images of live/dead cell viability assay in neurons. FIG. 8B shows quantification data of dead cells per square millimeter.

[0020] FIGs. 9A and 9B show differential expression analysis on RNA content of skin EVs. Volcano and heat map plots presenting differential gene content in Evs of 3xTg-AD

compared with B6129SF2/J at 23 weeks (A) 3xTg-AD 23 weeks compared with withB6129SF2/J 23 weeks and 3xTg-AD 10 weeks compared with B6129SF2/J 10 weeks (B) B6129SF2/J 23 weeks compared with B6129SF2/J 10 weeks and 3xTg-AD 23 weeks compared with 3xTg-AD 10 weeks.

[0021] FIGs. 10A and 10B show Top Canonical Pathways (FIG. 10A) and Disease/Functions Pathways (FIG. 10B) among all differential expression comparison groups show multiple dysregulated pathways in 3xTg-AD mice compared with controls.

DETAILED DESCRIPTION

[0022] Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to particular embodiments described, and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

[0023] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

[0024] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described.

[0025] All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication

provided could be different from the actual publication dates that may need to be independently confirmed.

[0026] As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

[0027] Embodiments of the present disclosure will employ, unless otherwise indicated, techniques of chemistry, biology, and the like, which are within the skill of the art.

[0028] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to perform the methods and use the probes disclosed and claimed herein. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C, and pressure is at or near atmospheric. Standard temperature and pressure are defined as 20 °C and 1 atmosphere.

[0029] Before the embodiments of the present disclosure are described in detail, it is to be understood that, unless otherwise indicated, the present disclosure is not limited to particular materials, reagents, reaction materials, manufacturing processes, or the like, as such can vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting. It is also possible in the present disclosure that steps can be executed in different sequence where this is logically possible.

[0030] It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise.

Definitions

[0031] The term “subject” refers to any individual who is the target of administration or treatment. The subject can be a vertebrate, for example, a mammal. Thus, the subject can be a human or veterinary patient. The term “patient” refers to a subject under the treatment of a clinician, e.g., physician.

[0032] The term “therapeutically effective” refers to the amount of the composition used is of sufficient quantity to ameliorate one or more causes or symptoms of a disease or

disorder. Such amelioration only requires a reduction or alteration, not necessarily elimination.

[0033] The term "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

[0034] The term "carrier" means a compound, composition, substance, or structure that, when in combination with a compound or composition, aids or facilitates preparation, storage, administration, delivery, effectiveness, selectivity, or any other feature of the compound or composition for its intended use or purpose. For example, a carrier can be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject.

[0035] The term "treatment" refers to the medical management of a patient with the intent to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder. This term includes active treatment, that is, treatment directed specifically toward the improvement of a disease, pathological condition, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, or disorder. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, or disorder.

[0036] The term "inhibit" refers to a decrease in an activity, response, condition, disease, or other biological parameter. This can include but is not limited to the complete ablation of the activity, response, condition, or disease. This may also include, for example, a 10% reduction in the activity, response, condition, or disease as compared to the native or control level. Thus, the reduction can be a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between as compared to native or control levels.

[0037] The term "polypeptide" refers to amino acids joined to each other by peptide bonds or modified peptide bonds, e.g., peptide isosteres, etc. and may contain modified amino acids other than the 20 gene-encoded amino acids. The polypeptides can be modified by either natural processes, such as post-translational processing, or by chemical

modification techniques which are well known in the art. Modifications can occur anywhere in the polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. The same type of modification can be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide can have many types of modifications. Modifications include, without limitation, acetylation, acylation, ADP-ribosylation, amidation, covalent cross-linking or cyclization, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of a phosphytidylinositol, disulfide bond formation, demethylation, formation of cysteine or pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristolyation, oxidation, pergylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, and transfer-RNA mediated addition of amino acids to protein such as arginylation. (See Proteins – Structure and Molecular Properties 2nd Ed., T.E. Creighton, W.H. Freeman and Company, New York (1993); Posttranslational Covalent Modification of Proteins, B.C. Johnson, Ed., Academic Press, New York, pp. 1-12 (1983)).

[0038] As used herein, the term “amino acid sequence” refers to a list of abbreviations, letters, characters or words representing amino acid residues. The amino acid abbreviations used herein are conventional one letter codes for the amino acids and are expressed as follows: A, alanine; B, asparagine or aspartic acid; C, cysteine; D aspartic acid; E, glutamate, glutamic acid; F, phenylalanine; G, glycine; H histidine; I isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine; Z, glutamine or glutamic acid.

[0039] The phrase “nucleic acid” as used herein refers to a naturally occurring or synthetic oligonucleotide or polynucleotide, whether DNA or RNA or DNA-RNA hybrid, single-stranded or double-stranded, sense or antisense, which is capable of hybridization to a complementary nucleic acid by Watson-Crick base-pairing. Nucleic acids can also include nucleotide analogs (e.g., BrdU), and non-phosphodiester internucleoside linkages (e.g., peptide nucleic acid (PNA) or thiodiester linkages). In particular, nucleic acids can include, without limitation, DNA, RNA, cDNA, gDNA, ssDNA, dsDNA or any combination thereof.

[0040] A “nucleotide” as used herein is a molecule that contains a base moiety, a sugar moiety, and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and sugar moieties creating an internucleoside linkage. The term “oligonucleotide” is sometimes used to refer to a molecule that contains two or more nucleotides linked together. The base moiety of a nucleotide can be adenine-9-yl (A),

cytosine-1-yl (C), guanine-9-yl (G), uracil-1-yl (U), and thymin-1-yl (T). The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a nucleotide is pentavalent phosphate. A non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate).

[0041] A nucleotide analog is a nucleotide that contains some type of modification to the base, sugar, and/or phosphate moieties. Modifications to nucleotides are well known in the art and would include, for example, 5-methylcytosine (5-me-C), 5 hydroxymethyl cytosine, xanthine, hypoxanthine, and 2-aminoadenine as well as modifications at the sugar or phosphate moieties.

[0042] Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

[0043] The term "vector" or "construct" refers to a nucleic acid sequence capable of transporting into a cell another nucleic acid to which the vector sequence has been linked. The term "expression vector" includes any vector, (e.g., a plasmid, cosmid or phage chromosome) containing a gene construct in a form suitable for expression by a cell (e.g., linked to a transcriptional control element). "Plasmid" and "vector" are used interchangeably, as a plasmid is a commonly used form of vector. Moreover, the invention is intended to include other vectors which serve equivalent functions.

[0044] The term "operably linked to" refers to the functional relationship of a nucleic acid with another nucleic acid sequence. Promoters, enhancers, transcriptional and translational stop sites, and other signal sequences are examples of nucleic acid sequences operably linked to other sequences. For example, operable linkage of DNA to a transcriptional control element refers to the physical and functional relationship between the DNA and promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA.

[0045] For purposes herein, the % sequence identity of a given nucleotides or amino acids sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given sequence C that has or comprises a certain % sequence identity to, with, or against a given sequence D) is calculated as follows:

[0046] 100 times the fraction W/Z,

[0047] where W is the number of nucleotides or amino acids scored as identical matches by the sequence alignment program in that program's alignment of C and D, and where Z is the total number of nucleotides or amino acids in D. It will be appreciated that where the length of sequence C is not equal to the length of sequence D, the % sequence identity of C to D will not equal the % sequence identity of D to C. Alignment for purposes of determining percent sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software.

[0048] By "specifically hybridizes" is meant that a probe, primer, or oligonucleotide recognizes and physically interacts (that is, base-pairs) with a substantially complementary nucleic acid (for example, a c-met nucleic acid) under high stringency conditions, and does not substantially base pair with other nucleic acids.

[0049] The term "stringent hybridization conditions" as used herein mean that hybridization will generally occur if there is at least 95% and preferably at least 97% sequence identity between the probe and the target sequence. Examples of stringent hybridization conditions are overnight incubation in a solution comprising 50% formamide, 5X SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared carrier DNA such as salmon sperm DNA, followed by washing the hybridization support in 0.1X SSC at approximately 65°C. Other hybridization and wash conditions are well known and are exemplified in Sambrook et al, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y. (1989), particularly chapter 11.

[0050] The "control elements" or "regulatory sequences" are those non-translated regions of the vector—enhancers, promoters, 5' and 3' untranslated regions—which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity.

[0051] A "promoter" is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A "promoter" contains core elements required for basic interaction of RNA polymerase and transcription factors and can contain upstream elements and response elements.

[0052] "Enhancer" generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' or 3' to the transcription unit. Furthermore, enhancers can be within an intron as well as within the coding sequence itself. They are usually between 10 and 300 bp in length, and they function in cis. Enhancers function to increase transcription from nearby promoters. Enhancers, like promoters, also

often contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression.

[0053] An "endogenous" enhancer/promoter is one which is naturally linked with a given gene in the genome. An "exogenous" or "heterologous" enhancer/promoter is one which is placed in juxtaposition to a gene by means of genetic manipulation (i.e., molecular biological techniques) such that transcription of that gene is directed by the linked enhancer/promoter.

Skin-Brain Axis

[0054] Alzheimer's Disease (AD) hallmark proteins, Amyloid Precursor Protein (APP), the Aβ peptide, and Tau are produced in multiple tissues outside the nervous system, suggesting that AD may be a systemic disease involving multiple organs. Outside the nervous system, amyloidosis has also been observed in the skin, the largest organ of the body (Feito-Rodríguez, M. et al. Actas Dermo-Sifiliográficas (English Edition) 2008 99:648–652), in which both Aβ peptides and Tau proteins are produced and amyloid aggregations are formed (Akerman, SC. et al. J. Alzheimers. Dis. 2019 69:463–478; Dugger, B. N. et al. J. Alzheimers. Dis. 2016 51:345–356). Furthermore, Aβ peptide and its oligomers are found in the brain and in the endothelium of blood vessels in the skin of AD patients and healthy subjects (Kucheryavykh, LY., et al. Int. J. Mol. Sci. 2018 19), and alterations in skin physiology are commonly observed in AD patients (Schreml, S., et al Exp. Dermatol. 2010 19:953–957). These alterations include metabolic changes (Jong, YJI., et al. FASEB J. 2003 17:2319–2321; Pani, A. et al. J. Alzheimers. Dis. 2009 18:829–841; Pitto, M. et al. Neurobiol. Aging 2005 26:833–838), changes in calcium metabolism and proliferation (Peterson, C., Proc. Natl. Acad. Sci. U. S. A. 1986 83:7999–8001), and reduction in vascular function (Kálmán, J. et al. Int. J. Geriatr. Psychiatry 2002 17:371–374), indicating the existence of a skin-brain axis connected through physiological and pathological mechanisms (Clos, AL., et al. Front. Neurol. 2012 3:5).

[0055] Previous findings indicate that circulating Aβ peptides synthesized in peripheral cells are able to cross the blood-brain barrier (Zlokovic, BV. et al. Biophys. Res. Commun. 1994 205:1431–1437), initiate Aβ-dependent neuropathologies and induce neuronal damage (Bu, X.-L. et al. Mol. Psychiatry 2018 23:1948–1956) through a prion-like mechanism (Jucker, M. & Walker, LC. Nature 2013 501:45–51). Moreover, another set of studies demonstrate that APP processing occurs intracellularly via the endocytic pathway (Rajendran, L. et al. Proc. Natl. Acad. Sci. U. S. A. 2006 103:11172–11177), and Aβ peptides are released as cargo inside extracellular vesicles capable of a neuron to neuron

transmission of the toxic Aβ peptides (Sardar Sinha, M. et al. Acta Neuropathol. 2018 136:41–56). Extracellular Vesicles (EVs), recently emerged as key players in intercellular communication by the transport of functional cargo such as DNA, RNA, and proteins (Iraci, N., et al. Int. J. Mol. Sci. 2016 17:171). Most cell types release EVs and in the skin EVs release has been characterized in the context of cell-cell communication, wound healing and cutaneous disorders (Terlecki-Zaniewicz, L. et al. J. Invest. Dermatol. 2019 139:2425–2436.e5). Disclosed herein is the characterization of a transcriptome of skin-derived EVs from the 3xTg-AD triple transgenic mouse model of AD, confirming the presence of mRNA of APP and Tau and how these molecules can be transferred to neurons via cellular uptake.

[0056] Therefore, as disclosed herein, the skin can dispatch signals to the brain in the form of exosomes, and exosomes derived from the skin of Alzheimer's Disease (AD) subjects contain neurotoxic cargo that could potentially be impacting the progression of this disease. This is a paradigm-shifting concept in AD, referred to herein as a "skin-brain axis." Disclosed herein are methods of using this phenomenon to diagnose neurological disease by assaying skin-derived exosomes from the subject, as well as methods of treating a subject with a neurological disease by modifying skin-derived exosomes with therapeutic cargo that will be delivered to the brain of the subject.

Diagnostic

[0057] Therefore, disclosed herein is a method for diagnosing a neurodegenerative disease in a subject that involves isolating skin-derived exosomes from the subject and assaying the exosomes for the presence of one or more biomarkers. I

[0058] Therefore, disclosed herein is a method for diagnosing a neurodegenerative disease in a subject that involves isolated exosomes from the subject and assaying the exosomes for the presence of one or more biomarkers. In some embodiments, the biomarker is S100A8, S100A9, MAPK13, or a combination thereof. In some embodiments, the biomarker is APP (NC_000021.9), Aβ, or Tau, or a combination thereof.

[0059] . In some embodiments, the biomarker is MAPT (NC_000017.11), ADAMTS9 (NC_000003.12), AKAP5 (NC_000014.9), AQP1 (NC_000007.14), ARC (NC_000008.11), CAMK2A (NC_000005.10), CASP4 (NC_000011.10), CLEC3B (NC_000003.12), FAAH (NC_000001.11), GRIN1 (NC_000009.12), HDAC9 (NC_000007.14), MAP2K3 (NC_000017.11), S100A8 (NC_000001.11), S100A9 (NC_000001.11), S100B (NC_000021.9), SLC16A6 (NC_000017.11), SLC1A3 (NC_000005.10), SLC25A4 (NC_000004.12), SLC30A3 (NC_000002.12), SLC38A2 (NC_000012.12), SLC39A3 (NC_000019.10), or any combination thereof.

[0060] In some embodiments, the biomarker is Rn45s, Mylpf, Neb, Rps3a1, Acta1, Atp2a1, Ckm, Ttn, Rnu11, Myh2, Xirp2, Lars2, Tnnc2, Dpt, Eno3, Elovl4, Des, Nr1d1, Tpm2, Tnnt3, Jph2, Smtnl1, Fbn1, Fmod, Serpina3j, Tnni2, Cmya5, Hspb6, Malat1, S100a9, Slc25a4, Trdn, Hfe2, Mybpc1, Casq1, Ryr1, Car3, Clec3b, Gas6, Fbxo40, Slc38a2, Myo18b, BC100530, Lor, Kdm6b, Myh6, Akr1e1, Cacna1s, Ogn, Spon2, Fhl1, Icam1, Myoc, Rnase2b, Mb, Mxd1, Gbp2b, Dhcr24, Mybph, Igfbp6, Alpk3, Gltp, Paqr7, Krt6b, Nr1d2, Dkk2, Igfn1, Hydin, Gm13177, Mypn, Cdsn, Myl1, Casc1, Dbp, Clca2, Tcea3, Anxa3, Hrc, L2hgdh, Spp1, Arid5b, Tpm1, Pcf11, Scarna2, Col3a1, Pttg1, Asb2, Krt6a, Dsc3, Trim54, Baiap3, 2310002L09Rik, Tgfb2, Il1b, Cilp, Pfkm, Cdr1, Gm13483, Scn10a, Slc17a7, Myh4, Camk2a, Cxcl2, Pclo, Mt1, Tnfaip3, Cntn2, Klhl31, Mstn, Dsp, Pcolce2, Fam207a, Tnnt1, Asic1, Tagap, Asb5, Fxyd2, Srl, Ldb3, Ryr3, Nrap, Mybpc2, Gse1, Abra, Ildr1, Smyd1, Hmox1, Obscn, Grin1, Stk36, S100a8, Zfp560, Fsd2, Polq, Thbs4, Gsdma, Krt80, 1-Mar, Fibin, Krt7, Pik3cg, Bcan, Flnc, Ndufv2, Tacstd2, Myoz1, Zxdb, Il11ra1, Neurl1a, Rbfox1, Cdh1, Fam83g, Snora23, Plet1, Zfp799, Taf15, Cstf1, 3425401B19Rik, Larp7, Slfn4, Gm13375, Ppp1r3a, Mmp15, A1cf, Chst3, F630111L10Rik, Gbp11, Gm6583, Lrguk, Mcidas, Rnf182, Syt2, Tsks, Ugt1a1, Mgme1, Dnah8, Dock10, Lama2, Arntl, Mitf, Fuca2, Kif9, Myl2, Adamts9, Jak2, Fndc5, S100b, Ces1d, Pygm, Dnah5, Ncan, Ppp1r3f, Hcar2, Nov, Ugcg, Arc, Tcf7l1, Tmem182, Ddhd1, A130010J15Rik, Hps3, Rlim, Hdac9, Nalcn, Fbxo17, Helb, Cd93, Cyp2b23, Gdpd3, Slfn10-ps, Trim72, Abat, Nup37, Zc3h12c, Kat2b, Agpat6, Vma21, Syn1, Pitrm1, Igfbp2, Pvalb, Nfkbiz, Hist1h2ab, Actn3, Myo5b, Dsc1, Sowahc, Sptbn2, Dna2, Eomes, Otog, Sike1, Ush1g, Muc19, Mroh4, Rbm46, Rimbp3, Gcnt2, Myh7, Cxcl14, Tep1, Myom2, Stfa3, Chil3, Sema7a, Vsx2, Nfkb1, Ovol1, Acss1, Zfp719, Prr12, Myom3, Arrdc4, H19, Dmpk, Sik1, Elovl6, Gspt2, Radil, Mfng, Atp6v1b2, Etv3, Rnasek, Schip1, Epha2, 2310003H01Rik, Saa3, Mfap4, Slc38a3, Stfa1, Mmp25, Zgrf1, Epha4, Mdm2, Lphn3, Arid2, Map2k3, Tgfbr1, Ckap2, Rilpl1, Srgn, Duox1, Cadps2, Wdr8, Vav2, Gna15, Tef, Ifit3, Limch1, Mmp17, Rasgrp3, B3glct, Pnpla1, Sfi1, Mgl2, Rad9a, Rap1gap, Utp23, Tbl1x, Wdr60, Casp4, Rab11fip1, Rbm20, Ptpn9, Snora64, Nrip1, Glp1r, 9530051G07Rik, Neurod1, Sh3gl3, Tex16, BC068157, Dennd6b, Setmar, Gtf2ird2, Shank3, Gm7120, Atp8b3, Shisa4, Sqle, Klhl41, Tmem198, 1810013L24Rik, Slc1a3, Tsc22d2, Emp2, F3, Hmga2-ps1, Xirp1, Rabl2, Speg, Mon1a, Dtna, Agap3, Phactr4, Piezo1, Spta1, Foxp2, Atp2b4, Crtc2, Gpr34, Lama5, Cdh5, Hrnr, Islr2, Plk2, Celsr1, Snord15a, Nhlrc1, St3gal3, Mapk13, Scarna13, Ccl4, Spint2, Jpx, Ankrd26, Efna1, Ggct, H2-T23, Myot, Pcca, Cacna2d2, Kpna7, Msh5, Batf, Lgals4, Fbxo30, Synpo2, 4930563E22Rik, C730027H18Rik, Fbxo27, Foxa2, Lrriq1, Npy6r, Pld5, Pth2r, Rab3c, Scgb3a2, Slc17a3, Slc6a7, Spata31d1d, Srrm4os, Tigd4, Tmco5, Vwa7, Gpr56, Adssl1, Pard6b, Usp53, Itch, Col24a1, Vipr1, Gm13178, Lrrc39,

Cntnap1, Myh3, Slfn9, Slc16a6, 4930431P03Rik, 9130204L05Rik, Ghsr, Icos, Slc26a3, Trappc3l, Unc5d, Pim1, Agtr1a, Tie1, Dancr, Phkb, Ubl3, Irf1, Smtn, Plk3, Efnb2, Abca8b, Lgals6, Tmppe, Pde4d, Clec2i, Gba, Brwd1, Smpx, Sprr2h, Fermt1, Chd3os, 5-Mar, Pkia, Spock2, Rhebl1, Col22a1, Amot, Pde4b, 9030624J02Rik, Pecam1, Gsto1, Gadl1, Lep, Gabra3, Kdm2b, Fndc9, Alpk2, Ptgs2, Tob2, Ccdc61, Fgf10, Scn4a, AU018091, Sumf1, Mir6516, Abca12, Sdr16c5, Txlnb, Snord22, Palm3, Itsn2, Pmm1, Coq10b, Aga, Selp, Mfap5, Slc16a13, Gtf2h3, Zswim4, Greb1, Gna12, Ddit4l, Cuedc2, Mrpl44, Gm15800, Ldlrad1, Misp, Nphp4, Cd14, Pfkfb1, Slc41a3, Herc4, Cadm4, Scarf2, Ckmt2, C030039L03Rik, Fkbp5, Wnk2, Hmces, Zbtb9, Hoxc10, Ccnl1, Cldn4, Ccl3, Eef2k, Irg1, Cpne7, Aqp1, A930013F10Rik, Exo1, Csf3, Dsc2, Trpm5, Fbxw17, Tmem150b, Ggps1, Bhlhb9, Hs1bp3, Fam102a, Pcyox1l, Fnip2, Zfp637, Cpt1b, Il31ra, C920009B18Rik, Klk7, Sp140, Rnf122, Adamts17, Irf6, Podxl, Klb, Dusp1, Dus1l, Slc2a4, Pgr, Snora26, D430019H16Rik, Fbxl15, Adamtsl4, Sbf1, Ccdc71l, Slc39a3, Col4a4, Rasl10b, Sspo, Tmf1, C030006K11Rik, Fundc2, Hccs, Olfml2a, Nppb, Plaur, Pld3, Hhipl1, Dusp13, Gpr171, Igf2bp3, Sfxn4, Adss, Tollip, Lmtk2, Sele, Nup62, Dusp5, Csrp3, Akap5, Myo1a, Onecut2, Clec11a, Ssc5d, Faah, Per2, Rfxap, Zfp131, Ippk, Endov, Top3b, Szt2, Zbtb16, Aacs, BC024139, Stx11, Stambpl1, Pcdh1, Trim45, Ttll8, Il2rg, Serpina3h, Sptbn4, Alox8, Lmod3, Arf2, Fosl1, Yae1d1, H2-Q4, Slc16a12, Stoml1, Epdr1, Bcl9l, Bpifc, Nuak1, Primpol, Abcb1b, Abca17, Abcc6, Cfi, Slc30a3, Tmem151b, Mylk2, Pde12, Lsr, C530008M17Rik, Nap1l5, Nos2, P4ha3, E330033B04Rik, Myo5c, 4931406P16Rik, Ugt1a7c, Dedd2, Cacna1b, 2810417H13Rik, Acvr2b, Hbegf, Fbxw9, Mfap3, Al661453, Fdx1, Ankrd55, Imp3, Grhl3, Map3k11, Hdc, Dennd3, Gpd1l, Akap1, R3hcc1, Procr, Adhfe1, 3-Sep, AY512915, Catsperg2, Cngb3, Dnaaf1, Fam132b, Fbxw18, Fuom, Gm5878, Oprl1, Slc6a13, Eef1a2, Alkbh1, Ccdc175, Clec4g, Kazald1, Ttbk1, Snora44, Acot6, Ralgds, Naa35, Gipc1, Ugt1a2, Ugt1a5, Ugt1a9, Fcer2a, Gp49a, Fiz1, Angptl7, Anks1b, Edn2, Fank1, Fgf6, Gm13032, Kbtbd8, Kcnq2, Mrgprx2, Fv1, Rab1b, Rasip1, Alox12b, or any combination thereof, including any 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 61, 62, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77. 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241,

242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 420, 430, 440, 441, 442, 443, 444, 445, 456, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 661, 662, 664, 665, or 666 of these biomarkers.

In some embodiments, the biomarker is a gene involved in Alzheimer's Disease, such as *Abat, Adamts9, Adss, Akap5, Alox12b, Ankrd55, Aqp1, Arc, Asb2, Atp6v1b2, Batf, Cadps2, Camk2a, Casp4, Ccl3, Ccl4, Cd14, Cd93, Ces1d, Cfi, Chst3, Clec3b, Cpne7, Cpt1b, Crtc2, Cxcl14, Dhcr24, Dsc2, Dtna, Dusp1, Eef2k, Elovl6, Epha2, Epha4, F3, Faah, Fbxo27, Fbxw9, Fkbp5, Fndc5, Fndc9, Gabra3, Gba, Gcnt2, Ggct, Ggps1, Grin1, Gsto1, Hdac9, Hmox1, Icam1, Igfbp2, Igfbp6, Il1b, Il31ra, Irf1, Itsn2, Kif9, Lama5, Ldb3, Lep, Lor, Lrrc39, Malat1, Map2k3, Mfap4, Mfng, Mybpc2, Myo5b, Myot, Nalcn, Nap1l5, Nfkb1, Nfkbiz, Nhlrc1, Nos2, Ogn, Pclo, Pcyox1l, Pecam1, Per2, Pfkm, Pgr, Piezo1, Pik3cg, Pim1, Plaur, Pld3, Plk2, Pmm1, Ptgs2, Rab3c, Rbfox1, Rhebl1, Rnase2b, S100a8, S100a9, S100b, Selp, Slc16a6, Slc1a3, Slc25a4, Slc30a3, Slc38a2, Slc39a3, Smtn, Sowahc, Spon2, Spp1, St3gal3, Stk36, Stoml1, Stx11, Tacstd2, Tbl1x, Tcf7l1, Tep1, Tgfb2, Tgfbr1, Tmem198, Tnfaip3, Tob2, Trim45, Trim54, Unc5d, Vipr1, Zbtb16, Zc3h12c*, or any combination thereof.

[0061] In some embodiments, the method involves assaying the exosomes for the regulation of one or more pathways related to neurodegenerative disease. For example, the method can involve assaying for calcium signaling, HMGB1 signaling, IL-6 signaling, and IL-8 signaling. In some embodiments, the method involves assaying the exosomes for the regulation of one or more pathways related to immune and inflammatory responses. For example, the method can involve assaying accumulation of neutrophils, migration of myeloid cells, activities of IL-6 and IL-8, or any combination thereof.

[0062] For example, the method can involve assaying for dysregulation of any pathway associated with a brain disorder, disease, or injury. For example, the pathway can be a Neuroinflammation Signaling Pathway, GP6 Signaling Pathway, Natural Killer Cell Signaling, HMGB1 Signaling, IL-6 Signaling, Actin Cytoskeleton Signaling, ILK Signaling, Factors Promoting Cardiogenesis in Vertebrates, IL-8 Signaling, Xenobiotic Metabolism AHR Signaling Pathway, IL-15 Production, Hepatic Fibrosis Signaling Pathway, Xenobiotic Metabolism General Signaling Pathway, Osteoarthritis Pathway, LXR/RXR Activation, Type I Diabetes Mellitus Signaling, Netrin Signaling, Dendritic Cell Maturation, Thyroid Hormone Metabolism II (via Conjugation and/or Degradation), Melatonin Degradation I, Nicotine Degradation II, Superpathway of Melatonin Degradation, Antioxidant Action of Vitamin C, TREM1 Signaling, Nicotine Degradation III, Cardiac Hypertrophy Signaling (Enhanced), Protein Kinase A Signaling, HIF1α Signaling, Mouse Embryonic Stem Cell Pluripotency, Gαi Signaling, Retinol Biosynthesis, Insulin Secretion Signaling Pathway, Calcium Signaling, HOTAIR Regulatory Pathway, Endothelin-1 Signaling, Xenobiotic Metabolism PXR Signaling Pathway, Semaphorin Neuronal Repulsive Signaling Pathway, Pancreatic Adenocarcinoma Signaling, Serotonin Degradation, Acute Phase Response Signaling, Toll-like Receptor Signaling, iNOS Signaling, Synaptogenesis Signaling Pathway, IL-17A Signaling in Airway Cells, p38 MAPK Signaling, Cardiac Hypertrophy Signaling, STAT3 Pathway, Th2 Pathway, Endocannabinoid Neuronal Synapse Pathway, Leukocyte Extravasation Signaling, Intrinsic Prothrombin Activation Pathway, Gα12/13 Signaling, PEDF Signaling, MIF Regulation of Innate Immunity, IL-23 Signaling Pathway, Paxillin Signaling, ErbB Signaling, Crosstalk between Dendritic Cells and Natural Killer Cells, Xenobiotic Metabolism CAR Signaling Pathway, Phospholipase C Signaling, CD40 Signaling, PKCθ Signaling in T Lymphocytes, AMPK Signaling, Regulation Of The Epithelial Mesenchymal Transition By Growth Factors Pathway, RhoA Signaling, GNRH Signaling, Colorectal Cancer Metastasis Signaling, Acetone Degradation I (to Methylglyoxal), Xanthine and Xanthosine Salvage, Glutathione-mediated Detoxification, Guanine and Guanosine Salvage I, Docosahexaenoic Acid (DHA) Signaling, Adenine and Adenosine Salvage I, Histamine Biosynthesis, Acetate Conversion to

Acetyl-CoA, Role of IL-17A in Arthritis, VDR/RXR Activation, Tight Junction Signaling, Glucocorticoid Receptor Signaling, Thyroid Hormone Biosynthesis, Aldosterone Signaling in Epithelial Cells, Superpathway of Cholesterol Biosynthesis, MSP-RON Signaling Pathway, Adenine and Adenosine Salvage III, Purine Nucleotides De Novo Biosynthesis II, Bladder Cancer Signaling, Antigen Presentation Pathway, Phospholipases, Arsenate Detoxification I (Glutaredoxin), Creatine-phosphate Biosynthesis, Cholesterol Biosynthesis I, Methylglyoxal Degradation III, Systemic Lupus Erythematosus Signaling, IL-10 Signaling, Th1 and Th2 Activation Pathway, Ephrin A Signaling, Prostanoid Biosynthesis, Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza, Calcium Transport I, IL-17 Signaling, Xenobiotic Metabolism Signaling, Eicosanoid Signaling, Iron homeostasis signaling pathway, Atherosclerosis Signaling, Triacylglycerol Degradation, Oncostatin M Signaling, Differential Regulation of Cytokine Production in Macrophages and T Helper Cells by IL-17A and IL-17F, Geranylgeranyldiphosphate Biosynthesis, Circadian Rhythm Signaling, Adenosine Nucleotides Degradation II, Estrogen-mediated S-phase Entry, The Visual Cycle, Apelin Cardiac Fibroblast Signaling Pathway, cAMP-mediated signaling, Communication between Innate and Adaptive Immune Cells, Purine Ribonucleosides Degradation to Ribose-1-phosphate, GABA Receptor Signaling, Cholesterol Biosynthesis III (via Desmosterol), Histidine Degradation VI, Epithelial Adherens Junction Signaling, Granulocyte Adhesion and Diapedesis, Role of IL-17A in Psoriasis, Zymosterol Biosynthesis, Choline Biosynthesis III, Heme Degradation, Bupropion Degradation, Purine Nucleotides Degradation II (Aerobic), Agranulocyte Adhesion and Diapedesis, Epoxysqualene Biosynthesis, Phagosome Maturation, Differential Regulation of Cytokine Production in Intestinal Epithelial Cells by IL-17A and IL-17F, Trans, trans-farnesyl Diphosphate Biosynthesis, Role of Tissue Factor in Cancer, MIF-mediated Glucocorticoid Regulation, Apelin Adipocyte Signaling Pathway, BAG2 Signaling Pathway, DNA damage-induced 14-3-3σ Signaling, Regulation of the Epithelial-Mesenchymal Transition Pathway, L-carnitine Biosynthesis, Estrogen Biosynthesis, RAR Activation, Cholesterol Biosynthesis II (via 24,25-dihydrolanosterol), Germ Cell-Sertoli Cell Junction Signaling, Hepatic Fibrosis / Hepatic Stellate Cell Activation, 4-aminobutyrate Degradation I, eNOS Signaling, Retinoate Biosynthesis I, IL-7 Signaling Pathway, Fatty Acid α-oxidation, Role of IL-17F in Allergic Inflammatory Airway Diseases, Cellular Effects of Sildenafil (Viagra), Graft-versus-Host Disease Signaling, nNOS Signaling in Skeletal Muscle Cells, Anandamide Degradation, Apelin Liver Signaling Pathway, Vitamin-C Transport, or any combination thereof.

[0063] In some aspects, the method is an immunoassay. Immunoassays, in their most simple and direct sense, are binding assays involving binding between antibodies and

antigen. Many types and formats of immunoassays are known and all are suitable for detecting the disclosed biomarkers. Examples of immunoassays are enzyme linked immunosorbent assays (ELISAs), radioimmunoassays (RIA), radioimmune precipitation assays (RIPA), immunobead capture assays, Western blotting, dot blotting, gel-shift assays, Flow cytometry, protein arrays, multiplexed bead arrays, magnetic capture, *in vivo* imaging, fluorescence resonance energy transfer (FRET), and fluorescence recovery/localization after photobleaching (FRAP/ FLAP).

[0064] In general, immunoassays involve contacting a sample suspected of containing a molecule of interest (such as the disclosed biomarkers) with an antibody to the molecule of interest or contacting an antibody to a molecule of interest (such as antibodies to the disclosed biomarkers) with a molecule that can be bound by the antibody, as the case may be, under conditions effective to allow the formation of immunocomplexes. Contacting a sample with the antibody to the molecule of interest or with the molecule that can be bound by an antibody to the molecule of interest under conditions effective and for a period of time sufficient to allow the formation of immune complexes (primary immune complexes) is generally a matter of simply bringing into contact the molecule or antibody and the sample and incubating the mixture for a period of time long enough for the antibodies to form immune complexes with, i.e., to bind to, any molecules (e.g., antigens) present to which the antibodies can bind. In many forms of immunoassay, the sample-antibody composition, such as a tissue section, ELISA plate, dot blot or Western blot, can then be washed to remove any non-specifically bound antibody species, allowing only those antibodies specifically bound within the primary immune complexes to be detected.

[0065] Immunoassays can include methods for detecting or quantifying the amount of a molecule of interest (such as the disclosed biomarkers or their antibodies) in a sample, which methods generally involve the detection or quantitation of any immune complexes formed during the binding process. In general, the detection of immunocomplex formation is well known in the art and can be achieved through the application of numerous approaches. These methods are generally based upon the detection of a label or marker, such as any radioactive, fluorescent, biological or enzymatic tags or any other known label.

[0066] In some aspects, the method comprises detecting mRNA biomarkers. A number of widely used procedures exist for detecting and determining the abundance of a particular mRNA in a total or poly(A) RNA sample. For example, specific mRNAs can be detected using Northern blot analysis, nuclease protection assays (NPA), in situ hybridization, or reverse transcription-polymerase chain reaction (RT-PCR). In some

embodiments, the method involves qRT-PCR, digital PCR, or in situ hybridization with molecular beacons or molecular flares/probes.

Modified Skin-Derived Extracellular vehicles (EVs)

[0067] Disclosed herein are methods of treating a subject with a neurological disease by modifying skin-derived exosomes with therapeutic cargo that will be delivered to the brain of the subject. In some embodiments, the method involves engineering the skin of the subject to produce therapeutic exosomes. In some embodiments, the method involves collecting skin-produced exosomes and loading them with therapeutic cargo.

Engineering Skin Cells to Produce Therapeutic EVs

[0068] Also disclosed are methods of reprogramming skin cells into EV-producing cells that involve delivering intracellularly into the skin cells a polynucleotide comprising nucleic acid sequences encoding therapeutic genes.

[0069] In some embodiments, this method involves transfecting the skin of the subject with an expression vector encoding an anti-Tau siRNA, miRNA, or any combination thereof. In some embodiments, this method involves transfecting the skin of the subject with an expression vector encoding one or more anti-inflammatory genes, such as siRNAs, or mRNAs that reduce glial cell activity.

[0070] In some embodiments, this method involves transfecting the skin of the subject with an expression vector encoding one or more vasculogenic factors, such as Etv2 (NM_001300974.2, NM_001304549.2, NM_014209.4), Foxc2 (NM_005251.3), Fli1 (NM_001167681.2, NM_001271010.1, NM_001271012.1, NM_002017.5), VEGFA (NM_001025366.3, NM_001025367.3, NM_001025368.3, NM_001025369.3, NM_001025370.3 NM_001033756.3, NM_001171622.2, NM_001171623.1, NM_001171624.1, NM_001171625.1, NM_001171626.1, NM_001171627.1, NM_001171628.1, NM_001171629.1, NM_001171630.1, NM_001204384.1, NM_001204385.2, NM_001287044.2, NM_001317010.1, NM_003376.6), VEGFB (NM_003377.5, NM_001243733.2), VEGFC (NM_005429.5), VEGFD (NM_004469.5), bFGF (NM_001361665.2, NM_002006.5), Sox17 (NM_022454.4), Oct4, Klf4 (NM_001314052.2, NM_004235.6), or any combination thereof.

[0071] In some embodiments, this method involves transfecting the skin of the subject with an expression vector encoding one or more neurogenic factors, such as Ascl1 (NM_004316.4), Ascl2 (NM_005170.3), Ascl3 (NM_020646.2), Ascl5 (NM_001270601.1), Neurog1 (NM_006161.3), Neurog2 (NM_024019.4), Neurog3 (NM_020999.4), Neurod1 (NM_002500.5), Neurod2 (NM_006160.4), Neurod4 (NM_021191.3), Neurod6

(NM_022728.4), Atoh1 (NM_005172.2), Atoh7 (NM_145178.4), Atoh8 (NM_032827.7), Myf5 (NM_005593.3), Ptf1a (NM_178161.3), Brn3c (NM_002700.3), Brn3a (NM_006237.4), Brn3b (NM_004575.3), Brn1 (NM_006236.3), Brn2 (NM_005604.4), Brn4 (NM_000307.5), Oct4 (NM_001173531.2), Oct6 (NM_002699.4), Pit1 (NM_000306.4), Brn5 (NM_001330422.2), Myt1l (NM_001303052.2), Nurr1 (NM_006186.4), or any combination thereof.

[0072] In some embodiments, this method involves transfecting the skin of the subject with an expression vector encoding an anti-APP siRNA, miRNA, such as hsa-miR-106b-5p, hsa-miR-101-3p, hsa-miR-520c-3p, hsa-miR-106a-5p, hsa-miR-20a-5p, hsa-miR-17-5p, hsa-miR-15a-5p, hsa-miR-130a-3p, hsa-let-7d-5p, hsa-let-7a-5p, hsa-miR-16-5p, hsa-miR-144-3p, hsa-miR-4422, hsa-let-7f-1-3p, hsa-let-7a-3p, hsa-let-7b-3p, hsa-miR-98-3p, hsa-miR-380-3p, hsa-miR-6835-3p, hsa-miR-4772-5p, hsa-miR-101-3p, hsa-miR-4719, hsa-miR-520f-3p, hsa-miR-3908, hsa-miR-4269, hsa-miR-323a-3p, hsa-miR-6715b-5p, hsa-miR-153-3p, hsa-miR-4495, hsa-miR-4786-5p, hsa-miR-3911, hsa-miR-6085, hsa-miR-6813-5p, or any combination thereof.

[0073] In some embodiments, this method involves transfecting the skin of the subject with an expression vector encoding an anti-MAPT siRNA, miRNA, such as hsa-miR-34c-5p, hsa-miR-657, hsa-miR-4728-5p, hsa-miR-3978, or any combination thereof.

[0074] In some embodiments, this method involves transfecting the skin of the subject with an expression vector encoding an anti-Inflammatory gene, such as *PPARγ* (NM_001330615.4, NM_001354666.3, NM_001354667.3, NM_001354668.2, NM_001354669.2, NM_001354670.2, NM_001374261.3, NM_001374262.3, NM_001374263.2, NM_001374264.2, NM_001374265.1, NM_001374266.1, NM_005037.7, NM_015869.5, NM_138711.6, NM_138712.5), *Il-10* (NM_000572.3, NM_001382624.1), *Il-27* (NM_145659.3), *Trem2* (NM_001271821.2, NM_018965.4), *IkBα* (NM_020529.3), *Sirt1* (NM_001142498.1, NM_001314049.1, NM_012238.5), or any combination thereof.

[0075] In some embodiments, this method involves transfecting the skin of the subject with an expression vector encoding an siRNA or miRNA targeting a pro-inflammatory gene. In some embodiments, the miRNA can be an anti-$P2X_4R$ miRNA, such as hsa-miR-335-5p, hsa-miR-106b-5p, or hsa-miR-20a-5p. In some embodiments, the miRNA can be an anti-TLR4 miRNA, such as hsa-let-7i-5p, hsa-miR-146a-5p, hsa-miR-335-5p, hsa-miR-146b-5p, hsa-let-7b-5p, hsa-miR-448, or hsa-miR-3924. In some embodiments, the miRNA can be an anti-CX3CR1 miRNA, such as hsa-miR-296-3p, hsa-miR-1227-3p, hsa-miR-4261, or hsa-miR-147b-5p. In some embodiments, the miRNA can be an anti-IL-1β miRNA, such as hsa-

miR-204-5p, hsa-miR-21-5p, hsa-miR-887-3p, hsa-miR-24-3p, hsa-miR-106a-5p, hsa-miR-877-3p, hsa-miR-5692a, hsa-miR-5688, or hsa-miR-495-3p.

[0076] In some embodiments, the nucleic acid sequences are present in non-viral vectors. In some embodiments, the nucleic acid sequences are operably linked to an expression control sequence. In other embodiments the nucleic acids are operably linked to two or more expression control sequences.

[0077] A variety of methods are known in the art and suitable for introduction of nucleic acid into a cell, including viral and non-viral mediated techniques. Examples of typical non-viral mediated techniques include, but are not limited to, electroporation, calcium phosphate mediated transfer, nucleofection, sonoporation, heat shock, magnetofection, liposome mediated transfer, microinjection, microprojectile mediated transfer (nanoparticles), cationic polymer mediated transfer (DEAE-dextran, polyethylenimine, polyethylene glycol (PEG) and the like) or cell fusion.

[0078] In some embodiments, after transfecting target cells, the cells can then pack the transfected genes (e.g. cDNA, miRNA, etc...) into EVs, which can then induce other skin cells to form EV-producing cells. Therefore, also disclosed is a method of reprogramming skin cells into EV-producing cells that involves exposing the somatic cell with an extracellular vesicle produced from a cell containing or expressing the disclosed therapeutic genes.

[0079] Therefore, disclosed are methods of reprogramming skin cells into EV-producing cells that involve exposing the skin cells to extracellular vesicles (EVs) isolated from cells expressing or containing exogenous polynucleotides comprising one or more nucleic acid sequences encoding the disclosed therapeutic genes. EVs secreted by the donor cells can then collected from the culture medium. These EVs can then be administered to the skin cells to reprogram them into insulin-producing cells. In some embodiments, the donor cells can be any cell from the subject able to produce EVs, including (but not limited to) skin cells (e.g., fibroblasts, keratinocytes, skin stem cells), adipocytes, dendritic cells, peripheral blood mononuclear cells (PBMC), pancreatic cells (e.g., ductal epithelial cells), liver cells (e.g., hepatocytes), immune cells (e.g., T cells, macrophages, myeloid derived suppressor cells).

[0080] Disclosed herein are compositions and methods for reprogramming skin cells into EV-producing cells both *in vitro* and *in vivo* that can be used to treat neurological diseases.

[0081] Exosomes and microvesicles are EVs that differ based on their process of biogenesis and biophysical properties, including size and surface protein markers. Exosomes are homogenous small particles ranging from 40 to 150 nm in size and they are

normally derived from the endocytic recycling pathway. In endocytosis, endocytic vesicles form at the plasma membrane and fuse to form early endosomes. These mature and become late endosomes where intraluminal vesicles bud off into an intra-vesicular lumen. Instead of fusing with the lysosome, these multivesicular bodies directly fuse with the plasma membrane and release exosomes into the extracellular space. Exosome biogenesis, protein cargo sorting, and release involve the endosomal sorting complex required for transport (ESCRT complex) and other associated proteins such as Alix and Tsg101. In contrast, microvesicles, are produced directly through the outward budding and fission of membrane vesicles from the plasma membrane, and hence, their surface markers are largely dependent on the composition of the membrane of origin. Further, they tend to constitute a larger and more heterogeneous population of extracellular vesicles, ranging from 150 to 1000 nm in diameter. However, both types of vesicles have been shown to deliver functional mRNA, miRNA and proteins to recipient cells.

[0082] In some embodiments, the polynucleotides are delivered to the somatic cells, or the donor cells for EVs, intracellularly via a gene gun, a microparticle or nanoparticle suitable for such delivery, transfection by electroporation, three-dimensional nanochannel electroporation, a tissue nanotransfection device, a liposome suitable for such delivery, or a deep-topical tissue nanoelectroinjection device. In some embodiments, a viral vector can be used. However, in other embodiments, the polynucleotides are not delivered virally.

[0083] Electroporation is a technique in which an electrical field is applied to cells in order to increase permeability of the cell membrane, allowing cargo (e.g., reprogramming factors) to be introduced into cells. Electroporation is a common technique for introducing foreign DNA into cells.

[0084] Tissue nanotransfection allows for direct cytosolic delivery of cargo (e.g., reprogramming factors) into cells by applying a highly intense and focused electric field through arrayed nanochannels, which benignly nanoporates the juxtaposing tissue cell members, and electrophoretically drives cargo into the cells.

[0085] In order to express a polypeptide or functional nucleic acid, the nucleotide coding sequence may be inserted into appropriate expression vector. Therefore, also disclosed is a non-viral vector comprising a polynucleotide comprising nucleic acid sequences disclosed herein, wherein the nucleic acid sequences are operably linked to an expression control sequence. In some embodiments, the nucleic acid sequences are operably linked to a single expression control sequence. In other embodiments, the nucleic acid sequences are operably linked to two or more separate expression control sequences.

[0086] Methods to construct expression vectors containing genetic sequences and appropriate transcriptional and translational control elements are well known in the art. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described in Sambrook et al., Molecular Cloning, A Laboratory Manual (Cold Spring Harbor Press, Plainview, N.Y., 1989), and Ausubel et al., Current Protocols in Molecular Biology (John Wiley & Sons, New York, N.Y., 1989).

[0087] Expression vectors generally contain regulatory sequences necessary elements for the translation and/or transcription of the inserted coding sequence. For example, the coding sequence is preferably operably linked to a promoter and/or enhancer to help control the expression of the desired gene product.

[0088] Promoters used in biotechnology are of different types according to the intended type of control of gene expression. They can be generally divided into constitutive promoters, tissue-specific or development-stage-specific promoters, inducible promoters, and synthetic promoters.

[0089] Constitutive promoters direct expression in virtually all tissues and are largely, if not entirely, independent of environmental and developmental factors. As their expression is normally not conditioned by endogenous factors, constitutive promoters are usually active across species and even across kingdoms. Examples of constitutive promoters include CMV, EF1a, SV40, PGK1, Ubc, Human beta actin, and CAG.

[0090] Tissue-specific or development-stage-specific promoters direct the expression of a gene in specific tissue(s) or at certain stages of development. For plants, promoter elements that are expressed or affect the expression of genes in the vascular system, photosynthetic tissues, tubers, roots and other vegetative organs, or seeds and other reproductive organs can be found in heterologous systems (e.g. distantly related species or even other kingdoms) but the most specificity is generally achieved with homologous promoters (i.e. from the same species, genus or family). This is probably because the coordinate expression of transcription factors is necessary for regulation of the promoter's activity.

[0091] The performance of inducible promoters is not conditioned to endogenous factors but to environmental conditions and external stimuli that can be artificially controlled. Within this group, there are promoters modulated by abiotic factors such as light, oxygen levels, heat, cold and wounding. Since some of these factors are difficult to control outside an experimental setting, promoters that respond to chemical compounds, not found naturally in the organism of interest, are of particular interest. Along those lines, promoters that

respond to antibiotics, copper, alcohol, steroids, and herbicides, among other compounds, have been adapted and refined to allow the induction of gene activity at will and independently of other biotic or abiotic factors.

[0092] The two most commonly used inducible expression systems for research of eukaryote cell biology are named Tet-Off and Tet-On. The Tet-Off system makes use of the tetracycline transactivator (tTA) protein, which is created by fusing one protein, TetR (tetracycline repressor), found in Escherichia coli bacteria, with the activation domain of another protein, VP16, found in the Herpes Simplex Virus. The resulting tTA protein is able to bind to DNA at specific TetO operator sequences. In most Tet-Off systems, several repeats of such TetO sequences are placed upstream of a minimal promoter such as the CMV promoter. The entirety of several TetO sequences with a minimal promoter is called a tetracycline response element (TRE), because it responds to binding of the tetracycline transactivator protein tTA by increased expression of the gene or genes downstream of its promoter. In a Tet-Off system, expression of TRE-controlled genes can be repressed by tetracycline and its derivatives. They bind tTA and render it incapable of binding to TRE sequences, thereby preventing transactivation of TRE-controlled genes. A Tet-On system works similarly, but in the opposite fashion. While in a Tet-Off system, tTA is capable of binding the operator only if not bound to tetracycline or one of its derivatives, such as doxycycline, in a Tet-On system, the rtTA protein is capable of binding the operator only if bound by a tetracycline. Thus the introduction of doxycycline to the system initiates the transcription of the genetic product. The Tet-On system is sometimes preferred over Tet-Off for its faster responsiveness.

[0093] In some embodiments, the nucleic acid sequences disclosed herein are operably linked to the same expression control sequence. Alternatively, internal ribosome entry sites (IRES) elements can be used to create multigene, or polycistronic, messages. IRES elements are able to bypass the ribosome scanning model of 5' methylated Cap dependent translation and begin translation at internal sites. IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, creating polycistronic messages. By virtue of the IRES element, each open reading frame is accessible to ribosomes for efficient translation. Multiple genes can be efficiently expressed using a single promoter/enhancer to transcribe a single message.

[0094] Disclosed are non-viral vectors containing one or more polynucleotides disclosed herein operably linked to an expression control sequence. Examples of such non-viral vectors include the oligonucleotide alone or in combination with a suitable protein,

polysaccharide or lipid formulation. Non-viral methods present certain advantages over viral methods, with simple large scale production and low host immunogenicity being just two. Previously, low levels of transfection and expression of the gene held non-viral methods at a disadvantage; however, recent advances in vector technology have yielded molecules and techniques with transfection efficiencies similar to those of viruses.

[0095] Examples of suitable non-viral vectors include, but are not limited to pIRES-hrGFP-2a, pCMV6, pMAX, pCAG, pAd-IRES-GFP, and pCDNA3.0.

[0096] The compositions disclosed can be used therapeutically in combination with a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

Modified Skin-Derived EVs

[0097] Also disclosed are methods of collecting skin-produced exosomes and loading them with therapeutic cargo. The disclosed EVs can in some embodiments be any vesicle that can be sereted by a cell. Cells secrete extracellular vesicles (EVs) with a broad range of diameters and functions, including apoptotic bodies (1–5 μm), microvesicles (100–1000 nm in size), and vesicles of endosomal origin, known as exosomes (50–150 nm).

[0098] The disclosed extracellular vesicles may be prepared by methods known in the art. For example, the disclosed extracellular vesicles may be prepared by expressing in a eukaryotic cell an mRNA that encodes the cell-targeting ligand. In some embodiments, the cell also expresses an mRNA that encodes a therapeutic cargo. The mRNA for the cell-targeting ligand and the therapeutic cargo may be expressed from vectors that are transfected into suitable production cells for producing the disclosed EVs. The mRNA for the cell-targeting ligand and the therapeutic cargo may be expressed from the same vector (e.g., where the vector expresses the mRNA for the cell-targeting ligand and the therapeutic cargo from separate promoters), or the mRNA for the cell-targeting ligand and the therapeutic cargo may be expressed from separate vectors. The vector or vectors for expressing the mRNA for the cell-targeting ligand and the therapeutic cargo may be packaged in a kit designed for preparing the disclosed extracellular vesicles.

[0099] Suitable carriers and their formulations are described in Remington: The Science and Practice of Pharmacy (19th ed.) ed. A.R. Gennaro, Mack Publishing Company,

Easton, PA 1995. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of composition being administered.

[0100] Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

[0101] Pharmaceutical compositions may include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

[0102] Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

[0103] Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

[0104] Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable..

[0105] Some of the compositions may potentially be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

[0106] The herein disclosed compositions, including pharmaceutical composition, may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. For example, the disclosed compositions can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally. The compositions may be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, ophthalmically, vaginally, rectally, intranasally, topically or the like, including topical intranasal administration or administration by inhalant.

Therapeutic cargo

[0107] The disclosed extracellular vesicles may be loaded with a therapeutic agent, where the extracellular vesicles deliver the agent to a target cell. Suitable therapeutic agents include but are not limited to therapeutic drugs (e.g., small molecule drugs), therapeutic proteins, and therapeutic nucleic acids (e.g., therapeutic RNA). In some embodiments, the disclosed extracellular vesicles comprise a therapeutic RNA (also referred to herein as a "cargo RNA").

[0108] For example, in some embodiments the fusion protein containing the cell-targeting motif also includes an RNA-domain (e.g., at a cytosolic C-terminus of the fusion protein) that binds to one or more RNA-motifs present in the cargo RNA in order to package the cargo RNA into the extracellular vesicle, prior to the extracellular vesicles being secreted from a cell. As such, the fusion protein may function as both of a "cell-targeting protein" and a "packaging protein." In some embodiments, the packaging protein may be referred to as extracellular vesicle-loading protein or "EV-loading protein."

[0109] In some embodiments, the cargo RNA is an miRNA, shRNA, mRNA, ncRNA, sgRNA or any combination thereof. For example, in some embodiments, the anti-inflammatory agent is micro-RNA 146a. Other miRNAs have been reported to regulate the expression of key molecules responsible for M1-favoring glycolytic metabolism (*e.g., mRr9,miR127 and miR155).*

[0110] The cargo RNA of the disclosed extracellular vesicles may be of any suitable length. For example, in some embodiments the cargo RNA may have a nucleotide length of at least about 10 nt, 20 nt, 30 nt, 40 nt, 50 nt, 100 nt, 200 nt, 500 nt, 1000 nt, 2000 nt, 5000 nt, or longer. In other embodiments, the cargo RNA may have a nucleotide length of no more than about 5000 nt, 2000 nt, 1000 nt, 500 nt, 200 nt, 100 nt, 50 nt, 40 nt, 30 nt, 20 nt, or 10 nt. In even further embodiments, the cargo RNA may have a nucleotide length within a range of these contemplated nucleotide lengths, for example, a nucleotide length between a range of about 10 nt-5000 nt, or other ranges. The cargo RNA of the disclosed extracellular vesicles may be relatively long, for example, where the cargo RNA comprises an mRNA or another relatively long RNA.

[0111] In some embodiments, the therapeutic cargo is a membrane-permeable pharmacological compound that is loaded into the EV after it is secreted by the cell. In some embodiments, the cargo is an anti-cancer agent that can cause apoptosis or pyroptosis of a targeted tumor cell. In some embodiments, the anti-cancer agent is a small molecule drug. For example, in some embodiments, the cargo is Ibrutinib. Additional examples of anti-cancer drugs or antineoplastics to be attached to the tumor targeting peptides described herein include, but are not limited to, aclarubicin, altretamine, aminopterin, amrubicin, azacitidine, azathioprine, belotecan, busulfan, camptothecin, capecitabine, carboplatin, carmofur, carmustine, chlorambucil, cisplatin, cladribine, clofarabine, cyclophosphamide, cytarabine, daunorubicin, decitabine, doxorubicin, epirubicin, etoposide, floxuridine, fludarabine, 5-fluorouracil, fluorouracil, gemcitabine, idarubicin, ifosfamide, irinotecan, mechlorethamine, melphalan, mercaptopurine, methotrexate, mitoxantrone, nedaplatin, oxaliplatin, paclitaxel, pemetrexed, pentostatin, pirarubicin, pixantrone, procarbazine, pyrimethamine raltitrexed, rubitecan, satraplatin, streptozocin, thioguanine, triplatin tetranitrate, teniposide, topotecan, tegafur, trimethoprim, uramustine, valrubicin, vinblastine, vincristine, vindesine, vinflunine, vinorelbine, and zorubicin.

[0112] To achieve loading of small RNAs into EVs, transfection-based approaches have been proposed. Other reports have shown that using vector- induced expression of small RNAs in cells, small RNA loading into EVs can be achieved. Alternatively, EV donor cells may be transfected with small RNAs directly. Incubation of tumor cells with

chemotherapeutic drugs is also another method to package drugs into EVs. To stimulate formation of drug-loaded EVs, cells are irradiated with ultraviolet light to induce apoptosis. Alternative approaches such as fusogenic liposomes also leads loading drugs into EVs.

[0113] In some embodiments, the therapeutic cargo is loaded into the EVs by diffusion via a concentration gradient.

Methods

[0114] Also contemplated herein are methods for using the disclosed EVs to treat a neurological disease. For example, the disclosed extracellular vesicles may be used for any injury, disease, or disorder of the brain by delivering a therapeutic gene or cargo. In some embodiments, the disclosed extracellular vesicles may be used to treat Spinal Cord Injury, Alzheimer's Disease, Amyotrophic Lateral Sclerosis, Ataxia, Cerebellar or Spinocerebellar Degeneration, Brain and Spinal Tumors, Cerebral Aneurysms, Epilepsy, Traumatic Brain Injury, Multiple Sclerosis, Parkinson's Disease, Stroke, Huntington's Disease, Autism Spectrum Disorder, Cerebral Palsy, Chronic Pain, Dementia With Lewy Bodies, Migraine, Niemann-Pick Disease, Frontotemporal Dementia, CADASIL, Spinocerebellar Degeneration and Atrophy, or any combination thereof.

[0115] The disclosed EVs may be administered to a subject by any suitable means. Administration to a human or animal subject may be selected from parenteral, intramuscular, intracerebral, intravascular, subcutaneous, or transdermal administration. Typically the method of delivery is by injection. Preferably the injection is intramuscular or intravascular (e.g. intravenous). A physician will be able to determine the required route of administration for each particular patient.

[0116] The EVs are preferably delivered as a composition. The composition may be formulated for parenteral, intramuscular, intracerebral, intravascular (including intravenous), subcutaneous, or transdermal administration. Compositions for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives. The EVs may be formulated in a pharmaceutical composition, which may include pharmaceutically acceptable carriers, thickeners, diluents, buffers, preservatives, and other pharmaceutically acceptable carriers or excipients and the like in addition to the EVs.

[0117] Parenteral administration is generally characterized by injection, such as subcutaneously, intramuscularly, or intravenously. Preparations for parenteral administration include sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets,

sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. The solutions may be either aqueous or nonaqueous.

[0118] If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof. Pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances. Examples of aqueous vehicles include sodium chloride injection, ringers injection, isotonic dextrose injection, sterile water injection, dextrose and lactated ringers injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Antimicrobial agents in bacteriostatic or fungistatic concentrations must be added to parenteral preparations packaged in multiple-dose containers which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include sodium chloride and dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcelluose, hydroxypropyl methylcellulose and polyvinylpyrrolidone.

[0119] Emulsifying agents include Polysorbate 80 (TWEEN® 80). A sequestering or chelating agent of metal ions include EDTA. Pharmaceutical carriers also include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles; and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment. The concentration of the pharmaceutically active compound is adjusted so that an injection provides an effective amount to produce the desired pharmacological effect. The exact dose depends on the age, weight and condition of the patient or animal as is known in the art.

[0120] The unit-dose parenteral preparations can be packaged in an ampoule, a vial or a syringe with a needle. All preparations for parenteral administration should be sterile, as is known and practiced in the art.

[0121] A therapeutically effective amount of composition is administered. The dose may be determined according to various parameters, especially according to the severity of the condition, age, and weight of the patient to be treated; the route of administration; and the required regimen. A physician will be able to determine the required route of

administration and dosage for any particular patient. Optimum dosages may vary depending on the relative potency of individual constructs, and can generally be estimated based on EC50s found to be effective in vitro and in vivo animal models. In general, dosage is from 0.01 mg/kg to 100 mg per kg of body weight. A typical daily dose is from about 0.1 to 50 mg per kg, preferably from about 0.1 mg/kg to 10 mg/kg of body weight, according to the potency of the specific construct, the age, weight and condition of the subject to be treated, the severity of the disease and the frequency and route of administration. Different dosages of the construct may be administered depending on whether administration is by intramuscular injection or systemic (intravenous or subcutaneous) injection.

[0122] Preferably, the dose of a single intramuscular injection is in the range of about 5 to 20 µg. Preferably, the dose of single or multiple systemic injections is in the range of 10 to 100 mg/kg of body weight.

[0123] Due to construct clearance (and breakdown of any targeted molecule), the patient may have to be treated repeatedly, for example once or more daily, weekly, monthly or yearly. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the construct in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy, wherein the construct is administered in maintenance doses, ranging from 0.01 mg/kg to 100 mg per kg of body weight, once or more daily, to once every 20 years.

[0124] Also disclosed herein is a method to reduce exosomal release from the skin to reducing trafficking to the brain. For example, in some embodiments, neutral sphingomyelinase inhibitor GW4869 can be applied topically and/or via intradermal injection to reduce skin-exosome release.

[0125] A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

EXAMPLES

Example 1: The Skin-Brain Axis in Alzheimer's Disease.

[0126] This Example seeks to elucidate the role of a potentially paradigm-shifting concept in Alzheimer's Disease (AD), the skin-brain axis, by evaluating the extent to which

exosomes shed by skin cells can remotely modulate the onset and/or progression of AD, and whether exosome engineering approaches can lead to novel therapeutic strategies against this disease (Fig. 1). The proposed studies thus target a number of focus areas of high interest in AD research and care, including defining the contribution of non-neuronal tissues to neurodegeneration, and elucidating disease mechanisms that may point to novel avenues for intervention. While significant progress has been made towards understanding the biochemistry, genetics and pathology of AD, there are still major knowledge gaps in the temporal sequence of cellular and molecular events underlying onset or progression (Selkoe D. Ann Intern Med, 140(8):627-638). This Example studies, for the first time, whether AD can be triggered, worsened, or even treated, via remote cues emanating from the skin. As such, the work proposed herein is fundamentally innovative and potentially transformative.

[0127] AD is the most common form of dementia currently affecting more than 5.8 million Americans, and expected to affect around 13.8 million by 2050 (Gaugler J, et al. ALZHEIMERS & DEMENTIA 2019, 15(3):321-387). AD is driven by the accumulation of plaques (i.e., aggregates of amyloid beta protein or Aβ) and tangles (i.e., aggregates of tau protein) within the brain (Small SA, et al. Neuron 2008, 60(4):534-542), which contribute to a profuse loss of synapses and neurons, partially mediated by inflammatory responses, eventually leading to a decline in mental abilities (Navarro Garrido V, et al. Frontiers in aging neuroscience 2018, 10:140; Mukhin V, et al. Neuroscience and Behavioral Physiology 2017, 47(5):508-516). Interestingly, the skin of AD patients has also been reported to harbor “deposits” of tau and amyloid (Bloom GS. JAMA neurology 2014, 71(4):505-508; Rodríguez-Leyva I, et al. J Mol Biomark Diagn S 2015, 6:005-010.4172; Jong Y-JI, et al. The FASEB journal 2003, 17(15):2319-2321; Okada A, et al. Dementia and Geriatric Cognitive Disorders 1994, 5(1):55-56). However, no clear functional role has been established for such deposits in the development or progression of AD. It was discovered in healthy mice that the skin can dispatch signals to the brain in the form of exosomes (Fig. 2), and that exosomes derived from the skin of mouse models of AD contain neurotoxic cargo that could potentially be impacting the progression of this disease (Fig. 3). Exosomes are cell-derived vesicles that play a crucial role in mediating cell-cell communications under both healthy and pathological conditions (S ELA, et al. Nat Rev Drug Discov 2013, 12(5):347-357; Ricklefs F, et al. Cancer Res 2016, 76(10):2876-2881; Hall J, et al. Cell Mol Neurobiol 2016, 36(3):417-427). It is hypothesize that: (1) exosomes derived from the skin could be cumulatively carrying neurotoxic cargo to the brain and contributing to the onset and/or progression of AD (i.e., skin-brain axis), and that (2) exosome engineering approaches can potentially be used as a novel therapeutic strategy against AD, using the skin as a “window” to the brain.

Example 2: Characterization of Extracellular Vesicles from the skin in the progression of Alzheimer's Disease.

[0128] Results

[0129] *Differential expression and pathway analyses of 3xTg-AD skin-derived* EV*sidentified changes in mRNA expression and pathways related to calcium signaling*

[0130] In order to characterize the neurodegenerative potential of skin-derived EVs within the context of AD, a broad characterization of differences in mRNA expression in 3xTg-AD and B6129SF2/J skin-derived EVs was conducted at 10 and 23 weeks of age (n=3). This was accomplished through the use of RNA-seq and subsequent differential expression analysis. The differentially expressed genes from each comparison were subsequently filtered for a log fold change ±1.5 and adjusted p-value of ≤ .05 (Figure 4). Ingenuity pathway analysis (IPA) was performed on resulting lists of differentially expressed genes in order to identify changes in canonical pathways and genes associated with specific diseases and/or functions (Figure 4).

[0131] *Differences in Extracellular Vesicles RNA-content as a function of Disease Genotype*

[0132] In order to determine the effect that the AD genotype has on changes in mRNA expression that occur within skin-derived EVs, the differential expression results from both 3xTg-AD 10 week vs. B6129SF2/J 10 weeks and 3xTg-AD 23 weeks vs. B6129SF2/J 23 weeks were taken into consideration. The 10 week comparison was considered in this case in order to evaluate the differences between 3xTg-AD and control skin-derived EVs at a relatively early time point in disease progression compared to 23 weeks. This was done in an effort to establish a "baseline" to compare with changes that occur with disease progression found in the 3xTg-AD 23 weeks vs. B6129SF2/J 23 weeks comparison. For the 3xTg-AD compared with B6129SF2/J at 10 weeks 91 genes were upregulated and 79 were downregulated. The 3xTg-AD 23 weeks vs. B6129SF2/J 23 weeks comparison was also considered here in order to evaluate how changes in mRNA expression occur at later stages in disease progression. The differential expression results revealed the upregulation of 240 genes and downregulation of 426 genes within this comparison (Table 1). In regards to pathways, calcium signaling showed the greatest amount of dysregulation within this comparison (-log p-value = 11.261) (Figure 10A, 10B). Furthermore, the dysregulation of calcium signaling pathways within the 3xTg-AD 23 weeks vs. B6129SF2/J 23 weeks group represented the greatest magnitude of dysregulation relative to other dysregulated pathways found among all comparison groups in this study. A total of ten genes implicated with calcium signaling were found to be downregulated in the 23 week 3xTg-AD mice. Of all the

genes found to be differentially expressed within this dataset, the upregulation of S100 calcium-binding protein A9 (S100A9) (Log2FoldChange = 6.155) was found to be of the greatest magnitude. The change in expression of this gene in particular is interesting given the fact that this upregulation is related to amyloid plaque accumulation within the AD brain (Wang, C. et al. Acta Neuropathol. 2014 127:507–522). Other dysregulated pathways in this comparison included actin cytoskeleton signaling, protein kinase A signaling, and RhoA signaling.

Table 1.

Name	Description	log2FoldChange	pValue	Padj
Rn45s	Rn45s	-2.01655	1.62E-13	1.23E-09
Mylpf	Mylpf	-3.05061	6.09E-12	2.30E-08
Neb	Neb	-2.29724	5.31E-11	1.33E-07
Rps3a1	Rps3a1	1.501043	8.98E-11	1.69E-07
Acta1	Acta1	-2.02599	5.13E-10	7.74E-07
Atp2a1	Atp2a1	-2.12498	8.98E-10	1.13E-06
Ckm	Ckm	-2.08923	1.25E-09	1.35E-06
Ttn	Ttn	-2.15305	3.94E-09	3.72E-06
Rnu11	Rnu11	-2.90544	1.79E-08	1.37E-05
Myh2	Myh2	-3.35743	1.81E-08	1.37E-05
Xirp2	Xirp2	-2.23579	2.04E-08	1.4E-05
Lars2	Lars2	-1.90255	3.81E-08	2.39E-05
Tnnc2	Tnnc2	-2.3053	1.55E-07	8.99E-05
Dpt	Dpt	-1.21916	2.47E-07	0.000131
Eno3	Eno3	-2.41246	2.61E-07	0.000131
Elovl4	Elovl4	1.185551	9.03E-07	0.000426
Des	Des	-1.70727	1.31E-06	0.000583
Nr1d1	Nr1d1	2.761581	2.47E-06	0.001037
Tpm2	Tpm2	-1.48427	4.36E-06	0.001675
Tnnt3	Tnnt3	-1.97564	4.44E-06	0.001675
Jph2	Jph2	-3.09823	8.63E-06	0.0031
Smtnl1	Smtnl1	-5.31377	1.35E-05	0.00464
Fbn1	Fbn1	-1.31336	1.59E-05	0.005109
Fmod	Fmod	-3.39621	1.62E-05	0.005109
Serpina3j	Serpina3j	1.61635	3.19E-05	0.009404
Tnni2	Tnni2	-1.82731	3.24E-05	0.009404
Cmya5	Cmya5	-1.94162	4.03E-05	0.011254
Hspb6	Hspb6	-2.6271	5.46E-05	0.014705
Malat1	Malat1	1.714919	6.25E-05	0.01627
S100a9	S100a9	6.155012	6.53E-05	0.016425

Slc25a4	Slc25a4	-1.13994	7.84E-05	0.018533
Trdn	Trdn	-2.19358	7.86E-05	0.018533
Hfe2	Hfe2	-6.1621	9.04E-05	1
Mybpc1	Mybpc1	-1.94276	9.48E-05	0.021676
Casq1	Casq1	-3.2052	0.000101	0.022364
Ryr1	Ryr1	-1.67613	0.000134	0.028896
Car3	Car3	-1.12579	0.000155	0.032546
Clec3b	Clec3b	-1.23964	0.000186	0.037767
Gas6	Gas6	-1.18604	0.00019	0.037767
Fbxo40	Fbxo40	-4.84696	0.000192	1
Slc38a2	Slc38a2	1.414986	0.000203	0.039271
Myo18b	Myo18b	-2.53696	0.000222	0.04188
BC100530	BC100530	4.041777	0.000267	0.047601
Lor	Lor	1.54947	0.000268	0.047601
Kdm6b	Kdm6b	1.296562	0.000271	0.047601
Myh6	Myh6	-4.42134	0.000293	0.050226
Akr1e1	Akr1e1	-3.81294	0.000346	0.058098
Cacna1s	Cacna1s	-2.48906	0.000355	0.058269
Ogn	Ogn	-1.49121	0.000445	0.071395
Spon2	Spon2	-2.5188	0.000468	0.073571
Fhl1	Fhl1	-1.20607	0.000503	0.077438
Icam1	Icam1	2.107226	0.000563	0.084925
Myoc	Myoc	-2.1158	0.000648	0.094972
Rnase2b	Rnase2b	5.186592	0.000654	0.094972
Mb	Mb	-2.06322	0.000691	0.096864
Mxd1	Mxd1	2.67428	0.000693	0.096864
Gbp2b	Gbp2b	-5.1882	0.000762	1
Dhcr24	Dhcr24	1.218208	0.000783	0.105503
Mybph	Mybph	-3.34594	0.000811	1
Igfbp6	Igfbp6	-1.19814	0.000837	0.110771
Alpk3	Alpk3	-2.44193	0.000884	0.114999
Gltp	Gltp	1.225324	0.000906	0.11589
Paqr7	Paqr7	3.599479	0.000953	0.118575
Krt6b	Krt6b	2.660958	0.001009	0.12263
Nr1d2	Nr1d2	2.299411	0.001024	0.12263
Dkk2	Dkk2	-1.78745	0.001045	0.123218
Igfn1	Igfn1	-4.42739	0.001103	1
Hydin	Hydin	-5.08037	0.001109	1
Gm13177	Gm13177	3.548479	0.001142	0.132556
Mypn	Mypn	-2.48279	0.001171	0.13315
Cdsn	Cdsn	1.781493	0.001199	0.13315
Myl1	Myl1	-1.60128	0.0012	0.13315

Casc1	Casc1	-5.71507	0.001204	1
Dbp	Dbp	4.922612	0.001309	0.142415
Clca2	Clca2	1.800484	0.001321	0.142415
Tcea3	Tcea3	-3.15101	0.00133	1
Anxa3	Anxa3	-1.43393	0.001353	0.143766
Hrc	Hrc	-3.07614	0.001398	1
L2hgdh	L2hgdh	-2.64147	0.00156	0.163548
Spp1	Spp1	5.06403	0.001686	0.174237
Arid5b	Arid5b	1.782864	0.001795	0.178688
Tpm1	Tpm1	-1.0815	0.0018	0.178688
Pcf11	Pcf11	1.713716	0.001829	0.179209
Scarna2	Scarna2	4.421738	0.001872	0.181073
Col3a1	Col3a1	-1.04751	0.001967	0.187904
Pttg1	Pttg1	-4.96692	0.002005	1
Asb2	Asb2	-3.12394	0.002026	0.188355
Krt6a	Krt6a	1.029594	0.002047	0.188355
Dsc3	Dsc3	1.241542	0.002163	0.196676
Trim54	Trim54	-2.82606	0.002201	0.197744
Baiap3	Baiap3	-4.27016	0.00223	1
2310002L09Rik	2310002L09Rik	-4.38627	0.002253	1
Tgfb2	Tgfb2	-2.60914	0.00233	0.203905
Il1b	Il1b	2.262838	0.002367	0.203905
Cilp	Cilp	-1.1313	0.002394	0.203905
Pfkm	Pfkm	-1.44563	0.002405	0.203905
Cdr1	Cdr1	-3.756	0.002405	1
Gm13483	Gm13483	-5.54958	0.002669	1
Scn10a	Scn10a	-5.54958	0.002669	1
Slc17a7	Slc17a7	-5.54958	0.002669	1
Myh4	Myh4	-2.46444	0.002741	0.229835
Camk2a	Camk2a	-4.22147	0.002761	1
Cxcl2	Cxcl2	3.921186	0.002785	0.230908
Pclo	Pclo	-3.39092	0.00285	0.233753
Mt1	Mt1	1.020204	0.002978	0.240546
Tnfaip3	Tnfaip3	1.309719	0.002996	0.240546
Cntn2	Cntn2	-4.19439	0.003052	1
Klhl31	Klhl31	-2.47059	0.003162	0.246059
Mstn	Mstn	-3.33973	0.003163	1
Dsp	Dsp	1.112861	0.003184	0.246059
Pcolce2	Pcolce2	-1.47185	0.003196	0.246059
Fam207a	Fam207a	-1.58746	0.003255	0.248099
Tnnt1	Tnnt1	-3.22037	0.003428	1
Asic1	Asic1	-5.57818	0.003442	1

Tagap	Tagap	-5.57818	0.003442	1
Asb5	Asb5	-2.45579	0.003446	0.260022
Fxyd2	Fxyd2	-3.39095	0.003481	1
Srl	Srl	-1.54532	0.003633	0.269433
Ldb3	Ldb3	-1.78926	0.003642	0.269433
Ryr3	Ryr3	-4.22302	0.003653	1
Nrap	Nrap	-1.52443	0.00375	0.273009
Mybpc2	Mybpc2	-1.44084	0.003775	0.273009
Gse1	Gse1	2.762572	0.003799	0.273009
Abra	Abra	-3.49618	0.00389	1
Ildr1	Ildr1	-4.89509	0.003899	1
Smyd1	Smyd1	-4.15977	0.003926	1
Hmox1	Hmox1	1.779002	0.003929	0.279708
Obscn	Obscn	-1.31582	0.003987	0.280198
Grin1	Grin1	-5.52872	0.00399	1
Stk36	Stk36	-5.52872	0.00399	1
S100a8	S100a8	3.31818	0.00401	0.280198
Zfp560	Zfp560	-3.17168	0.004098	1
Fsd2	Fsd2	-4.78529	0.004158	1
Polq	Polq	-3.36578	0.004316	1
Thbs4	Thbs4	-1.49677	0.004335	0.298691
Gsdma	Gsdma	4.478911	0.004644	1
Krt80	Krt80	1.815378	0.004816	0.327409
1-Mar	1-Mar	-3.61266	0.00485	1
Fibin	Fibin	-2.03958	0.004921	0.331529
Krt7	Krt7	-4.82646	0.004968	1
Pik3cg	Pik3cg	-2.62412	0.005037	0.336382
Bcan	Bcan	-4.73606	0.005324	1
Flnc	Flnc	-1.25649	0.005437	0.356777
Ndufv2	Ndufv2	-1.20956	0.005578	0.362495
Tacstd2	Tacstd2	1.169254	0.00562	0.362495
Myoz1	Myoz1	-1.97927	0.005686	0.36364
Zxdb	Zxdb	-3.2303	0.00569	1
Il11ra1	Il11ra1	-1.19363	0.006103	0.386975
Neurl1a	Neurl1a	-4.02742	0.006141	1
Rbfox1	Rbfox1	-4.02742	0.006141	1
Cdh1	Cdh1	1.218104	0.00646	0.405499
Fam83g	Fam83g	2.406272	0.006502	0.405499
Snora23	Snora23	2.277804	0.006696	0.410801
Plet1	Plet1	1.603181	0.006822	0.41518
Zfp799	Zfp799	-2.82483	0.006958	1
Taf15	Taf15	-1.04278	0.006962	0.420268

Cstf1	Cstf1	-2.05162	0.007177	0.426454
3425401B19Rik	3425401B19Rik	-4.0632	0.007332	1
Larp7	Larp7	-1.63099	0.00748	0.435798
Slfn4	Slfn4	3.67866	0.007554	0.435798
Gm13375	Gm13375	-1.40837	0.007566	0.435798
Ppp1r3a	Ppp1r3a	-2.38748	0.007904	0.448068
Mmp15	Mmp15	-1.99343	0.007957	0.448068
A1cf	A1cf	-5.32309	0.008058	1
Chst3	Chst3	-5.32309	0.008058	1
F630111L10Rik	F630111L10Rik	-5.32309	0.008058	1
Gbp11	Gbp11	-5.32309	0.008058	1
Gm6583	Gm6583	-5.32309	0.008058	1
Lrguk	Lrguk	-5.32309	0.008058	1
Mcidas	Mcidas	-5.32309	0.008058	1
Rnf182	Rnf182	-5.32309	0.008058	1
Syt2	Syt2	-5.32309	0.008058	1
Tsks	Tsks	-5.32309	0.008058	1
Ugt1a1	Ugt1a1	1.41649	0.008076	0.4514
Mgme1	Mgme1	-3.51674	0.008096	1
Dnah8	Dnah8	-3.06769	0.008214	1
Dock10	Dock10	-1.79807	0.008441	0.46834
Lama2	Lama2	-1.0285	0.008592	0.471436
Arntl	Arntl	-1.80777	0.008622	0.471436
Mitf	Mitf	2.031193	0.008762	0.474532
Fuca2	Fuca2	-2.05736	0.008804	0.474532
Kif9	Kif9	-4.5795	0.008967	1
Myl2	Myl2	-4.5795	0.008967	1
Adamts9	Adamts9	1.629535	0.008978	0.474686
Jak2	Jak2	1.332688	0.009181	0.474686
Fndc5	Fndc5	-4.5801	0.009197	1
S100b	S100b	-4.5801	0.009197	1
Ces1d	Ces1d	1.523836	0.009199	0.474686
Pygm	Pygm	-1.38063	0.009325	0.474686
Dnah5	Dnah5	-5.34894	0.009443	1
Ncan	Ncan	-5.34894	0.009443	1
Ppp1r3f	Ppp1r3f	-5.34894	0.009443	1
Hcar2	Hcar2	3.700267	0.009481	0.474686
Nov	Nov	-1.95145	0.009619	0.474686
Ugcg	Ugcg	1.437981	0.009731	0.474686
Arc	Arc	1.69328	0.009775	0.474686
Tcf7l1	Tcf7l1	2.545233	0.009783	0.474686
Tmem182	Tmem182	-4.62744	0.009826	1

Ddhd1	Ddhd1	-1.27582	0.009854	0.474686
A130010J15Rik	A130010J15Rik	4.250029	0.009929	1
Hps3	Hps3	-2.14534	0.009929	0.474686
Rlim	Rlim	1.218141	0.009939	0.474686
Hdac9	Hdac9	-3.44531	0.010138	1
Nalcn	Nalcn	-3.86922	0.010347	1
Fbxo17	Fbxo17	-2.77379	0.010422	1
Helb	Helb	-2.15889	0.010437	0.491963
Cd93	Cd93	2.239158	0.010496	0.491963
Cyp2b23	Cyp2b23	-2.76826	0.010627	1
Gdpd3	Gdpd3	3.533623	0.010832	0.501441
Slfn10-ps	Slfn10-ps	-2.3461	0.010877	1
Trim72	Trim72	-3.08128	0.010949	1
Abat	Abat	-3.07088	0.011126	1
Nup37	Nup37	-2.6722	0.01115	1
Zc3h12c	Zc3h12c	3.224777	0.011196	0.512034
Kat2b	Kat2b	1.513738	0.011482	0.520907
Agpat6	Agpat6	-1.19519	0.011583	0.520907
Vma21	Vma21	-1.49647	0.011597	0.520907
Syn1	Syn1	-4.05181	0.011635	1
Pitrm1	Pitrm1	-1.2312	0.011835	0.525516
Igfbp2	Igfbp2	-1.75395	0.011839	0.525516
Pvalb	Pvalb	-2.84838	0.012106	0.528142
Nfkbiz	Nfkbiz	1.146794	0.01219	0.528142
Hist1h2ab	Hist1h2ab	-1.91852	0.012196	0.528142
Actn3	Actn3	-2.19786	0.012226	0.528142
Myo5b	Myo5b	4.162	0.012229	1
Dsc1	Dsc1	1.447436	0.012282	0.528142
Sowahc	Sowahc	1.430243	0.012364	0.528142
Sptbn2	Sptbn2	1.542313	0.012458	0.528142
Dna2	Dna2	-3.17686	0.012544	1
Eomes	Eomes	-4.5388	0.012569	1
Otog	Otog	-4.5388	0.012569	1
Sike1	Sike1	-1.68526	0.012647	0.53018
Ush1g	Ush1g	-4.53946	0.012657	1
Muc19	Muc19	-3.84302	0.012823	1
Mroh4	Mroh4	-4.54073	0.012828	1
Rbm46	Rbm46	-4.54073	0.012828	1
Rimbp3	Rimbp3	-4.54073	0.012828	1
Gcnt2	Gcnt2	2.429269	0.012986	0.541132
Myh7	Myh7	-5.56409	0.012991	1
Cxcl14	Cxcl14	-1.1179	0.013051	0.541132

Tep1	Tep1	-1.39784	0.013279	0.544806
Myom2	Myom2	-1.6264	0.013284	0.544806
Stfa3	Stfa3	4.052464	0.01331	1
Chil3	Chil3	4.107292	0.013367	1
Sema7a	Sema7a	3.473088	0.013394	0.546317
Vsx2	Vsx2	-3.85519	0.013427	1
Nfkb1	Nfkb1	1.021191	0.0135	0.547695
Ovol1	Ovol1	1.695461	0.013727	0.553944
Acss1	Acss1	-2.20503	0.013733	1
Zfp719	Zfp719	-2.20503	0.013733	1
Prr12	Prr12	-1.24287	0.013922	0.555808
Myom3	Myom3	-2.21099	0.014012	1
Arrdc4	Arrdc4	1.428118	0.01403	0.555808
H19	H19	-1.19307	0.014068	0.555808
Dmpk	Dmpk	-1.54205	0.014308	0.558444
Sik1	Sik1	1.050553	0.014366	0.558444
Elovl6	Elovl6	1.1129	0.014431	0.558444
Gspt2	Gspt2	-3.37355	0.014536	1
Radil	Radil	-2.24283	0.014635	1
Mfng	Mfng	-2.01299	0.014714	0.566507
Atp6v1b2	Atp6v1b2	1.490241	0.014899	0.570528
Etv3	Etv3	1.418816	0.01497	0.570528
Rnasek	Rnasek	-1.46015	0.015284	0.572815
Schip1	Schip1	-2.06398	0.015419	0.572815
Epha2	Epha2	1.49251	0.015546	0.572815
2310003H01Rik	2310003H01Rik	3.995093	0.015609	1
Saa3	Saa3	3.600453	0.015624	0.572815
Mfap4	Mfap4	-1.14363	0.015827	0.57357
Slc38a3	Slc38a3	-3.3972	0.016002	1
Stfa1	Stfa1	3.37521	0.01611	0.5769
Mmp25	Mmp25	-2.78755	0.016181	1
Zgrf1	Zgrf1	-2.78755	0.016181	1
Epha4	Epha4	2.349317	0.016198	0.5769
Mdm2	Mdm2	1.427786	0.016251	0.5769
Lphn3	Lphn3	-2.10189	0.016412	0.57873
Arid2	Arid2	1.602695	0.01651	0.579463
Map2k3	Map2k3	1.182899	0.016708	0.583686
Tgfbr1	Tgfbr1	-1.2562	0.016795	0.584035
Ckap2	Ckap2	-2.06533	0.016899	1
Rilpl1	Rilpl1	-1.61429	0.016999	0.588149
Srgn	Srgn	1.808823	0.017069	0.588149
Duox1	Duox1	-2.44034	0.017082	1

Cadps2	Cadps2	-2.08184	0.017216	1
Wdr8	Wdr8	-2.43287	0.017249	1
Vav2	Vav2	3.955594	0.017574	1
Gna15	Gna15	-1.87521	0.017631	0.595922
Tef	Tef	1.436745	0.017734	0.595922
Ifit3	Ifit3	-1.48969	0.017767	0.595922
Limch1	Limch1	-1.43699	0.017769	0.595922
Mmp17	Mmp17	-2.97939	0.017891	1
Rasgrp3	Rasgrp3	-2.80942	0.018044	1
B3glct	B3glct	-1.90724	0.018131	0.602701
Pnpla1	Pnpla1	4.079731	0.018201	1
Sfi1	Sfi1	-1.75378	0.018213	0.602786
Mgl2	Mgl2	-1.17383	0.018533	0.610686
Rad9a	Rad9a	4.060476	0.018548	1
Rap1gap	Rap1gap	-3.34707	0.018561	1
Utp23	Utp23	-2.28598	0.018698	1
Tbl1x	Tbl1x	1.431094	0.019608	0.632581
Wdr60	Wdr60	-1.93055	0.019762	0.632581
Casp4	Casp4	3.328155	0.019764	0.632581
Rab11fip1	Rab11fip1	1.848593	0.019865	0.632581
Rbm20	Rbm20	-2.27054	0.019878	1
Ptpn9	Ptpn9	2.004286	0.019936	0.632581
Snora64	Snora64	2.448604	0.020094	0.634447
Nrip1	Nrip1	1.873151	0.020226	0.635942
Glp1r	Glp1r	-3.6841	0.020368	1
9530051G07Rik	9530051G07Rik	-4.34401	0.020416	1
Neurod1	Neurod1	-4.34401	0.020416	1
Sh3gl3	Sh3gl3	-4.34401	0.020416	1
Tex16	Tex16	-4.34401	0.020416	1
BC068157	BC068157	-4.34445	0.020601	1
Dennd6b	Dennd6b	-4.34445	0.020601	1
Setmar	Setmar	-4.34445	0.020601	1
Gtf2ird2	Gtf2ird2	-2.80274	0.020638	1
Shank3	Shank3	1.972941	0.020738	0.64932
Gm7120	Gm7120	-4.34526	0.020945	1
Atp8b3	Atp8b3	-4.3961	0.021016	1
Shisa4	Shisa4	-4.3961	0.021016	1
Sqle	Sqle	1.394828	0.02114	0.650452
Klhl41	Klhl41	-1.59091	0.021162	0.650452
Tmem198	Tmem198	-3.27054	0.021219	1
1810013L24Rik	1810013L24Rik	1.206171	0.021301	0.650452
Slc1a3	Slc1a3	2.008642	0.021385	0.650452

Tsc22d2	Tsc22d2	1.331276	0.021439	0.650452
Emp2	Emp2	1.028533	0.021489	0.650452
F3	F3	1.873621	0.02155	0.650452
Hmga2-ps1	Hmga2-ps1	-2.35729	0.021774	1
Xirp1	Xirp1	-1.5126	0.021848	0.656495
Rabl2	Rabl2	-2.69903	0.022077	1
Speg	Speg	-1.41565	0.022142	0.656495
Mon1a	Mon1a	3.783599	0.022223	1
Dtna	Dtna	-2.07759	0.022342	1
Agap3	Agap3	1.72541	0.022366	0.656495
Phactr4	Phactr4	1.794199	0.022398	0.656495
Piezo1	Piezo1	1.347808	0.022456	0.656495
Spta1	Spta1	-2.36328	0.022494	1
Foxp2	Foxp2	-2.3492	0.022496	1
Atp2b4	Atp2b4	1.165353	0.022514	0.656495
Crtc2	Crtc2	1.766244	0.022651	0.656495
Gpr34	Gpr34	-3.22816	0.022711	1
Lama5	Lama5	1.399448	0.022714	0.656495
Cdh5	Cdh5	1.671799	0.022787	0.656495
Hrnr	Hrnr	3.064058	0.022842	0.656495
Islr2	Islr2	-2.96566	0.022845	1
Plk2	Plk2	1.217302	0.022954	0.656495
Celsr1	Celsr1	1.48351	0.023099	0.656495
Snord15a	Snord15a	2.736879	0.023222	0.656495
Nhlrc1	Nhlrc1	-2.51226	0.023463	1
St3gal3	St3gal3	-1.93522	0.023605	0.66465
Mapk13	Mapk13	3.237848	0.023762	0.666563
Scarna13	Scarna13	3.345444	0.024075	0.670364
Ccl4	Ccl4	3.764646	0.024182	1
Spint2	Spint2	1.996497	0.024366	0.673514
Jpx	Jpx	-3.73683	0.024391	1
Ankrd26	Ankrd26	-2.49433	0.024427	1
Efna1	Efna1	3.767076	0.024515	1
Ggct	Ggct	2.084914	0.024539	0.674774
H2-T23	H2-T23	1.345586	0.024591	0.674774
Myot	Myot	-3.18173	0.024744	0.676526
Pcca	Pcca	-1.67413	0.02485	0.676954
Cacna2d2	Cacna2d2	-2.71639	0.025064	1
Kpna7	Kpna7	-3.84452	0.025112	1
Msh5	Msh5	-3.84452	0.025112	1
Batf	Batf	-2.91086	0.025266	1
Lgals4	Lgals4	3.909103	0.025318	1

Fbxo30	Fbxo30	1.684031	0.025329	0.677196
Synpo2	Synpo2	-1.24475	0.025335	0.677196
4930563E22Rik	4930563E22Rik	-5.06583	0.025432	1
C730027H18Rik	C730027H18Rik	-5.06583	0.025432	1
Fbxo27	Fbxo27	-5.06583	0.025432	1
Foxa2	Foxa2	-5.06583	0.025432	1
Lrriq1	Lrriq1	-5.06583	0.025432	1
Npy6r	Npy6r	-5.06583	0.025432	1
Pld5	Pld5	-5.06583	0.025432	1
Pth2r	Pth2r	-5.06583	0.025432	1
Rab3c	Rab3c	-5.06583	0.025432	1
Scgb3a2	Scgb3a2	-5.06583	0.025432	1
Slc17a3	Slc17a3	-5.06583	0.025432	1
Slc6a7	Slc6a7	-5.06583	0.025432	1
Spata31d1d	Spata31d1d	-5.06583	0.025432	1
Srrm4os	Srrm4os	-5.06583	0.025432	1
Tigd4	Tigd4	-5.06583	0.025432	1
Tmco5	Tmco5	-5.06583	0.025432	1
Vwa7	Vwa7	-5.06583	0.025432	1
Gpr56	Gpr56	1.238249	0.025463	0.677196
Adssl1	Adssl1	-1.70656	0.0256	0.677196
Pard6b	Pard6b	3.268984	0.025672	0.677196
Usp53	Usp53	3.14222	0.025725	0.677196
Itch	Itch	1.421224	0.025873	0.677196
Col24a1	Col24a1	-2.50778	0.025904	1
Vipr1	Vipr1	-2.70191	0.025919	1
Gm13178	Gm13178	-1.23643	0.025936	0.677196
Lrrc39	Lrrc39	-2.99663	0.026033	1
Cntnap1	Cntnap1	-3.6746	0.026048	1
Myh3	Myh3	-3.6746	0.026048	1
Slfn9	Slfn9	-2.22853	0.026208	1
Slc16a6	Slc16a6	-1.99641	0.026336	1
4930431P03Rik	4930431P03Rik	-5.08035	0.026407	1
9130204L05Rik	9130204L05Rik	-5.08035	0.026407	1
Ghsr	Ghsr	-5.08035	0.026407	1
Icos	Icos	-5.08035	0.026407	1
Slc26a3	Slc26a3	-5.08035	0.026407	1
Trappc3l	Trappc3l	-5.08035	0.026407	1
Unc5d	Unc5d	-5.08035	0.026407	1
Pim1	Pim1	1.132869	0.026447	0.685807
Agtr1a	Agtr1a	-2.00912	0.026539	0.685835
Tie1	Tie1	3.145369	0.027	0.693399

Dancr	Dancr	2.543538	0.027169	0.693399
Phkb	Phkb	-1.32101	0.027265	0.693399
Ubl3	Ubl3	1.277234	0.027447	0.693399
Irf1	Irf1	1.239416	0.027621	0.693399
Smtn	Smtn	1.552558	0.027743	0.693399
Plk3	Plk3	1.896446	0.027761	0.693399
Efnb2	Efnb2	1.333616	0.027784	0.693399
Abca8b	Abca8b	-1.52081	0.027843	0.693399
Lgals6	Lgals6	3.863529	0.027994	1
Tmppe	Tmppe	3.690236	0.028044	1
Pde4d	Pde4d	-1.43076	0.028053	0.694051
Clec2i	Clec2i	-3.627	0.028247	1
Gba	Gba	1.900757	0.028366	0.69741
Brwd1	Brwd1	1.15313	0.028373	0.69741
Smpx	Smpx	-2.23702	0.028509	1
Sprr2h	Sprr2h	3.844695	0.028562	1
Fermt1	Fermt1	-1.60246	0.028682	0.698988
Chd3os	Chd3os	3.693416	0.02879	1
5-Mar	5-Mar	-1.09371	0.028865	0.698988
Pkia	Pkia	-1.34705	0.028881	0.698988
Spock2	Spock2	-1.67084	0.028901	0.698988
Rhebl1	Rhebl1	-3.087	0.028944	1
Col22a1	Col22a1	-3.68719	0.029023	1
Amot	Amot	-1.57915	0.029055	0.70047
Pde4b	Pde4b	1.469652	0.029184	0.701139
9030624J02Rik	9030624J02Rik	2.149677	0.029268	0.701139
Pecam1	Pecam1	2.274054	0.02942	0.702354
Gsto1	Gsto1	2.580653	0.029505	0.702354
Gadl1	Gadl1	3.917891	0.029719	1
Lep	Lep	-1.18627	0.029802	0.704267
Gabra3	Gabra3	-1.83279	0.030075	1
Kdm2b	Kdm2b	-1.14038	0.030091	0.704267
Fndc9	Fndc9	3.778463	0.030118	1
Alpk2	Alpk2	-2.64799	0.030244	1
Ptgs2	Ptgs2	1.123744	0.030378	0.704267
Tob2	Tob2	1.061583	0.030405	0.704267
Ccdc61	Ccdc61	-2.16224	0.030464	1
Fgf10	Fgf10	-1.3767	0.030589	0.704267
Scn4a	Scn4a	-1.77772	0.030621	0.704267
AU018091	AU018091	-3.24906	0.03087	1
Sumf1	Sumf1	-1.32746	0.031002	0.704267
Mir6516	Mir6516	2.69143	0.031046	0.704267

Abca12	Abca12	2.429326	0.0311	0.704267
Sdr16c5	Sdr16c5	-1.70895	0.031203	0.704267
Txlnb	Txlnb	-1.70895	0.031203	0.704267
Snord22	Snord22	2.031569	0.031205	0.704267
Palm3	Palm3	-2.69503	0.031205	1
Itsn2	Itsn2	1.313373	0.031232	0.704267
Pmm1	Pmm1	-1.72852	0.031246	0.704267
Coq10b	Coq10b	2.524455	0.031396	0.704267
Aga	Aga	-2.15299	0.031421	1
Selp	Selp	2.510309	0.031429	0.704267
Mfap5	Mfap5	-1.21148	0.031444	0.704267
Slc16a13	Slc16a13	3.624454	0.031754	1
Gtf2h3	Gtf2h3	3.630426	0.031797	1
Zswim4	Zswim4	1.29712	0.031885	0.70765
Greb1	Greb1	-2.29316	0.032032	1
Gna12	Gna12	2.095262	0.032063	0.709514
Ddit4l	Ddit4l	-2.15929	0.032358	1
Cuedc2	Cuedc2	-1.40664	0.032499	0.712891
Mrpl44	Mrpl44	-1.9592	0.032725	1
Gm15800	Gm15800	1.324911	0.032725	0.715783
Ldlrad1	Ldlrad1	-3.70237	0.03275	1
Misp	Misp	-3.70237	0.03275	1
Nphp4	Nphp4	-3.12695	0.032815	1
Cd14	Cd14	1.611754	0.032964	0.717388
Pfkfb1	Pfkfb1	-3.18335	0.032993	1
Slc41a3	Slc41a3	-2.67082	0.033061	1
Herc4	Herc4	1.230762	0.033215	0.718441
Cadm4	Cadm4	-1.76013	0.033228	0.718441
Scarf2	Scarf2	-1.84297	0.033354	1
Ckmt2	Ckmt2	-2.99366	0.033391	1
C030039L03Rik	C030039L03Rik	-2.84183	0.033489	1
Fkbp5	Fkbp5	1.15811	0.033735	0.726063
Wnk2	Wnk2	2.423433	0.033773	0.726063
Hmces	Hmces	-2.06355	0.0339	1
Zbtb9	Zbtb9	-2.28349	0.034116	1
Hoxc10	Hoxc10	3.630434	0.034295	1
Ccnl1	Ccnl1	1.204192	0.034301	0.732532
Cldn4	Cldn4	2.256268	0.034497	0.732532
Ccl3	Ccl3	3.615042	0.034552	1
Eef2k	Eef2k	1.190443	0.034698	0.733415
Irg1	Irg1	3.039383	0.034796	1
Cpne7	Cpne7	-2.62108	0.034818	1

Aqp1	Aqp1	1.798313	0.034833	0.734209
A930013F10Rik	A930013F10Rik	-2.86912	0.034971	1
Exo1	Exo1	-2.86912	0.034971	1
Csf3	Csf3	3.106168	0.03499	0.734442
Dsc2	Dsc2	1.607391	0.03507	0.734442
Trpm5	Trpm5	-3.63423	0.035175	1
Fbxw17	Fbxw17	-2.09966	0.035232	1
Tmem150b	Tmem150b	-3.21964	0.035438	1
Ggps1	Ggps1	-1.6552	0.035505	0.738077
Bhlhb9	Bhlhb9	-1.76813	0.03559	1
Hs1bp3	Hs1bp3	3.636764	0.035676	1
Fam102a	Fam102a	1.246918	0.035839	0.742969
Pcyox1l	Pcyox1l	-3.05695	0.036333	1
Fnip2	Fnip2	1.469361	0.036498	0.754566
Zfp637	Zfp637	3.599414	0.036623	1
Cpt1b	Cpt1b	-2.54241	0.036726	1
Il31ra	Il31ra	-2.06238	0.036833	1
C920009B18Rik	C920009B18Rik	-2.78645	0.037169	1
Klk7	Klk7	1.22907	0.037199	0.759678
Sp140	Sp140	-1.4007	0.037249	0.759678
Rnf122	Rnf122	3.587858	0.03736	1
Adamts17	Adamts17	-2.83093	0.037523	1
Irf6	Irf6	1.064384	0.038411	0.770105
Podxl	Podxl	1.931629	0.038508	0.770105
Klb	Klb	3.542937	0.038629	1
Dusp1	Dusp1	1.305675	0.038639	0.770105
Dus1l	Dus1l	-1.46951	0.038656	0.770105
Slc2a4	Slc2a4	-1.74691	0.038679	0.770105
Pgr	Pgr	-3.53011	0.038919	0.771065
Snora26	Snora26	2.378358	0.039175	0.771065
D430019H16Rik	D430019H16Rik	-3.53117	0.039189	1
Fbxl15	Fbxl15	-2.42598	0.039203	1
Adamtsl4	Adamtsl4	-1.46202	0.039315	0.771065
Sbf1	Sbf1	1.072082	0.03934	0.771065
Ccdc71l	Ccdc71l	1.265438	0.039542	0.771163
Slc39a3	Slc39a3	-1.75205	0.039578	0.771163
Col4a4	Col4a4	-1.52264	0.039652	0.771163
Rasl10b	Rasl10b	-3.51489	0.039769	1
Sspo	Sspo	-1.81154	0.039774	1
Tmf1	Tmf1	1.057854	0.039792	0.771902
C030006K11Rik	C030006K11Rik	3.684524	0.039868	1
Fundc2	Fundc2	-1.0793	0.039927	0.772534

Hccs	Hccs	-1.4279	0.040294	0.777634
Olfml2a	Olfml2a	-3.48079	0.041139	1
Nppb	Nppb	-3.27851	0.041216	1
Plaur	Plaur	2.35689	0.041319	0.783703
Pld3	Pld3	1.395109	0.041376	0.783703
Hhipl1	Hhipl1	-3.01056	0.041516	1
Dusp13	Dusp13	-4.39025	0.041519	1
Gpr171	Gpr171	-4.39025	0.041519	1
Igf2bp3	Igf2bp3	-4.39025	0.041519	1
Sfxn4	Sfxn4	-4.39025	0.041519	1
Adss	Adss	1.318301	0.041548	0.783703
Tollip	Tollip	1.01268	0.041619	0.783703
Lmtk2	Lmtk2	1.442088	0.041759	0.783703
Sele	Sele	3.165771	0.041807	0.783703
Nup62	Nup62	1.365993	0.041908	0.783703
Dusp5	Dusp5	2.0537	0.042025	0.783703
Csrp3	Csrp3	-1.06994	0.042043	0.783703
Akap5	Akap5	-3.44167	0.042157	1
Myo1a	Myo1a	-3.44167	0.042157	1
Onecut2	Onecut2	-3.44167	0.042157	1
Clec11a	Clec11a	-2.34205	0.042259	1
Ssc5d	Ssc5d	-1.89547	0.042291	0.783703
Faah	Faah	2.957955	0.042309	0.783703
Per2	Per2	3.526256	0.04236	1
Rfxap	Rfxap	3.507042	0.042412	1
Zfp131	Zfp131	1.913723	0.042418	0.783703
Ippk	Ippk	2.15445	0.042477	0.783703
Endov	Endov	-1.84361	0.042571	1
Top3b	Top3b	-1.45725	0.042723	0.783703
Szt2	Szt2	-1.39192	0.042965	0.783703
Zbtb16	Zbtb16	1.040903	0.042986	0.783703
Aacs	Aacs	1.280801	0.04303	0.783703
BC024139	BC024139	-3.16878	0.043177	1
Stx11	Stx11	2.34188	0.043348	0.783703
Stambpl1	Stambpl1	-1.95341	0.043398	0.783703
Pcdh1	Pcdh1	1.596894	0.043516	0.783703
Trim45	Trim45	-3.40933	0.043535	1
Ttll8	Ttll8	-3.40933	0.043535	1
Il2rg	Il2rg	3.500525	0.043641	1
Serpina3h	Serpina3h	3.51072	0.043661	1
Sptbn4	Sptbn4	-2.68427	0.043699	1
Alox8	Alox8	-3.23664	0.043821	1

Lmod3	Lmod3	-3.23664	0.043821	1
Arf2	Arf2	1.577249	0.043936	0.787504
Fosl1	Fosl1	1.026545	0.044154	0.787665
Yae1d1	Yae1d1	3.490386	0.044193	1
H2-Q4	H2-Q4	1.396741	0.044804	0.791097
Slc16a12	Slc16a12	-2.20982	0.044862	1
Stoml1	Stoml1	-2.20982	0.044862	1
Epdr1	Epdr1	-1.66756	0.044972	0.791097
Bcl9l	Bcl9l	1.099934	0.044997	0.791097
Bpifc	Bpifc	1.943582	0.045046	0.791097
Nuak1	Nuak1	1.746204	0.045062	0.791097
Primpol	Primpol	-2.66591	0.045088	1
Abcb1b	Abcb1b	-1.30393	0.045114	0.791097
Abca17	Abca17	-4.12328	0.045115	1
Abcc6	Abcc6	-4.12328	0.045115	1
Cfi	Cfi	-4.12328	0.045115	1
Slc30a3	Slc30a3	-4.12328	0.045115	1
Tmem151b	Tmem151b	-4.12328	0.045115	1
Mylk2	Mylk2	-1.55802	0.045204	0.791097
Pde12	Pde12	1.64205	0.045289	0.791097
Lsr	Lsr	1.711094	0.045425	0.791632
C530008M17Rik	C530008M17Rik	-4.12315	0.045645	1
Nap1l5	Nap1l5	-4.12315	0.045645	1
Nos2	Nos2	-4.12315	0.045645	1
P4ha3	P4ha3	-4.12315	0.045645	1
E330033B04Rik	E330033B04Rik	-3.20835	0.045727	1
Myo5c	Myo5c	-2.65689	0.045778	1
4931406P16Rik	4931406P16Rik	1.567154	0.045841	0.793379
Ugt1a7c	Ugt1a7c	1.119735	0.046131	0.79471
Dedd2	Dedd2	2.284704	0.046233	0.79471
Cacna1b	Cacna1b	-3.05751	0.04639	1
2810417H13Rik	2810417H13Rik	3.518507	0.046436	1
Acvr2b	Acvr2b	-2.60996	0.046537	1
Hbegf	Hbegf	1.522238	0.046556	0.798442
Fbxw9	Fbxw9	3.50676	0.046881	1
Mfap3	Mfap3	3.517178	0.046954	1
AI661453	AI661453	1.576707	0.047182	0.80143
Fdx1	Fdx1	3.469752	0.047194	1
Ankrd55	Ankrd55	-3.04468	0.047228	1
Imp3	Imp3	1.995868	0.047231	0.80143
Grhl3	Grhl3	2.667311	0.047235	0.80143
Map3k11	Map3k11	1.298096	0.047347	0.80143

Hdc	Hdc	1.938371	0.047368	0.80143
Dennd3	Dennd3	3.535881	0.047741	1
Gpd1l	Gpd1l	-1.33149	0.047752	0.804153
Akap1	Akap1	-1.56552	0.047867	0.804153
R3hcc1	R3hcc1	-1.23952	0.048011	0.804153
Procr	Procr	1.87423	0.048062	0.804153
Adhfe1	Adhfe1	-2.29151	0.048167	1
3-Sep	3-Sep	-1.86308	0.048452	1
AY512915	AY512915	-4.07252	0.048462	1
Catsperg2	Catsperg2	-4.07252	0.048462	1
Cngb3	Cngb3	-4.07252	0.048462	1
Dnaaf1	Dnaaf1	-4.07252	0.048462	1
Fam132b	Fam132b	-4.07252	0.048462	1
Fbxw18	Fbxw18	-4.07252	0.048462	1
Fuom	Fuom	-4.07252	0.048462	1
Gm5878	Gm5878	-4.07252	0.048462	1
Oprl1	Oprl1	-4.07252	0.048462	1
Slc6a13	Slc6a13	-4.07252	0.048462	1
Eef1a2	Eef1a2	-1.60802	0.048748	0.804583
Alkbh1	Alkbh1	-1.61313	0.04883	0.804583
Ccdc175	Ccdc175	-4.07333	0.048882	1
Clec4g	Clec4g	-4.07333	0.048882	1
Kazald1	Kazald1	-4.07333	0.048882	1
Ttbk1	Ttbk1	-4.07333	0.048882	1
Snora44	Snora44	1.758293	0.048891	0.804583
Acot6	Acot6	-3.01994	0.048913	1
Ralgds	Ralgds	1.221898	0.048986	0.804583
Naa35	Naa35	-1.26159	0.049347	0.804583
Gipc1	Gipc1	1.541166	0.049387	0.804583
Ugt1a2	Ugt1a2	1.165772	0.049424	0.804583
Ugt1a5	Ugt1a5	1.165772	0.049424	0.804583
Ugt1a9	Ugt1a9	1.165772	0.049424	0.804583
Fcer2a	Fcer2a	-3.50917	0.049444	1
Gp49a	Gp49a	2.849744	0.049503	1
Fiz1	Fiz1	-1.32831	0.049551	0.804583
Angptl7	Angptl7	-4.07479	0.049663	1
Anks1b	Anks1b	-4.07479	0.049663	1
Edn2	Edn2	-4.07479	0.049663	1
Fank1	Fank1	-4.07479	0.049663	1
Fgf6	Fgf6	-4.07479	0.049663	1
Gm13032	Gm13032	-4.07479	0.049663	1
Kbtbd8	Kbtbd8	-4.07479	0.049663	1

Kcnq2	Kcnq2	-4.07479	0.049663	1
Mrgprx2	Mrgprx2	-4.07479	0.049663	1
Fv1	Fv1	-2.75313	0.049807	1
Rab1b	Rab1b	1.008347	0.049831	0.804583
Rasip1	Rasip1	2.065895	0.049874	0.804583
Alox12b	Alox12b	3.086733	0.049896	1

[0133] *Differences in Extracellular Vesicles RNA-content as a function of Age*

[0134] Differential expression and IPA analyses were performed on 3xTg-AD 23 weeks vs. 3xTg-AD 10 weeks and B6129SF2/J 23 weeks vs. B6129SF2/J 10 weeks to determine the effect that age has in both the AD and control mice. A total of 150 upregulated genes and 143 downregulated genes were found in 3xTg-AD 23 week vs. 3xTg-AD 10-week comparison (Figure 9B). Pathway analysis of these genes revealed the dysregulation of several pathways, including hepatic fibrosis, GP6 signaling, and apelin liver signaling. The comparison of B6129SF2/J 23 weeks vs. B6129SF2/J 10 weeks was performed to evaluate the changes in expression due to aging alone. The results revealed the upregulation of 98 genes, as well as the downregulation of 98 others (Table 2). Top related pathways that were identified include unfolded protein response, RhoA Signaling, and adrenomedullin signaling (Figure 10A, 10B).

Table 2.

Name	Description	log2FoldChange	pValue	Padj
Scap	Scap	-2.67827	2.71E-16	3.23E-13
Vaultrc5	Vaultrc5	-2.01653	2.16E-09	1.29E-06
Serpina3j	Serpina3j	-2.02098	8.04E-07	0.00032
Rps3a1	Rps3a1	-1.22472	5.22E-06	0.001559
Ttn	Ttn	1.213967	3.13E-05	0.0074
Elovl4	Elovl4	-1.05938	3.72E-05	0.0074
Psapl1	Psapl1	-1.04776	0.000358	0.057057
Ces1d	Ces1d	-2.31005	0.000382	0.057057
S100a9	S100a9	-5.66722	0.0004	1
Rnase2b	Rnase2b	-5.61867	0.000444	1
Mcl1	Mcl1	-1.14158	0.000569	0.075544
Tpm1	Tpm1	1.200706	0.000719	0.081237
Dhcr24	Dhcr24	-1.26664	0.00078	0.081237
Ctsd	Ctsd	-1.01856	0.000816	0.081237
Cxcl2	Cxcl2	-3.79001	0.001196	1
Akr1c18	Akr1c18	2.999493	0.001585	1

Snord22	Snord22	-1.38341	0.001834	0.156386
Serpina3b	Serpina3b	-1.14557	0.001992	0.158581
Rgs9	Rgs9	-1.0233	0.002814	0.20553
Hist1h2ab	Hist1h2ab	4.551693	0.002968	1
Acta1	Acta1	1.002455	0.003051	0.20553
Lphn3	Lphn3	5.126657	0.003063	1
Il1b	Il1b	-2.46363	0.003211	1
Neb	Neb	1.090694	0.003271	0.20553
Jph2	Jph2	3.144739	0.003293	1
Fbxo40	Fbxo40	5.082152	0.003565	1
Iigp1	Iigp1	-2.11099	0.003597	1
Gse1	Gse1	-3.10302	0.003768	1
Prpf40a	Prpf40a	1.883555	0.003801	1
Ly6g6c	Ly6g6c	-1.29149	0.003945	0.206967
Irf1	Irf1	-1.73793	0.004124	0.206967
Gbp2	Gbp2	-2.09345	0.004148	1
Slc38a2	Slc38a2	-1.31748	0.00416	0.206967
Anxa3	Anxa3	1.53754	0.005685	0.261053
Dbp	Dbp	-4.83447	0.0059	1
S100a8	S100a8	-3.29959	0.006723	1
Lars2	Lars2	1.290893	0.006765	0.295033
Cux1	Cux1	1.629415	0.007082	1
Zfp560	Zfp560	4.920765	0.007108	1
Actn3	Actn3	1.448642	0.007158	0.295033
Ces4a	Ces4a	-1.5667	0.007347	1
Mitf	Mitf	-2.3857	0.0085	1
Tgoln1	Tgoln1	-1.20235	0.008864	0.341407
Srgn	Srgn	-2.36225	0.009253	1
Mylpf	Mylpf	1.232371	0.010863	0.376635
Ryr1	Ryr1	1.229883	0.010966	0.376635
Igfn1	Igfn1	4.794133	0.011266	1
2310002L09Rik	2310002L09Rik	4.818736	0.011423	1
Xaf1	Xaf1	-3.87613	0.011565	1
Hmox1	Hmox1	-1.85346	0.012013	1
Col4a4	Col4a4	4.129727	0.01213	1
Mapk13	Mapk13	-3.79302	0.01226	1
B3glct	B3glct	4.139149	0.012501	1
Mroh2a	Mroh2a	4.772877	0.01252	1
Hcar2	Hcar2	-3.79597	0.012641	1
Elovl3	Elovl3	-1.60337	0.013158	1
Ggta1	Ggta1	2.387077	0.013822	1
Scarna2	Scarna2	-3.73829	0.014679	1

E030003E18Rik	E030003E18Rik	2.545239	0.014861	1
Mxd1	Mxd1	-2.41606	0.015368	1
Mb	Mb	2.193699	0.0154	1
Myot	Myot	1.983929	0.015701	1
Smtnl1	Smtnl1	2.843728	0.016347	1
Trdn	Trdn	1.960649	0.016729	1
Myh2	Myh2	1.546649	0.016762	1
Pnpla2	Pnpla2	-1.11759	0.01715	0.472193
Clca2	Clca2	-2.32086	0.017401	0.472193
Saa3	Saa3	-3.69364	0.017505	1
Baiap3	Baiap3	4.679702	0.017658	1
Prickle3	Prickle3	4.679702	0.017658	1
Mrgprg	Mrgprg	-4.37746	0.017778	1
Crip2	Crip2	1.08262	0.017862	0.473947
Kprp	Kprp	2.037065	0.017974	1
H2-T23	H2-T23	-1.67094	0.018654	1
Cmss1	Cmss1	4.71226	0.019045	1
Cdr1	Cdr1	4.654431	0.019229	1
Smad1	Smad1	1.777526	0.019968	1
Klf9	Klf9	-1.11282	0.02014	0.52276
L2hgdh	L2hgdh	3.342868	0.020452	1
Nr1d1	Nr1d1	-1.76272	0.020713	1
Manf	Manf	-1.44295	0.021053	1
Fmod	Fmod	2.260675	0.021854	1
Fam102a	Fam102a	-1.63857	0.022495	1
Mcpt4	Mcpt4	-1.61799	0.022791	1
Polq	Polq	4.627446	0.023	1
Ndufv2	Ndufv2	1.442308	0.02386	1
Glb1l2	Glb1l2	-2.65042	0.024039	1
Rnu11	Rnu11	2.291792	0.024268	0.568562
Cxcr2	Cxcr2	-4.27521	0.024309	1
Ccl4	Ccl4	-4.31817	0.024546	1
F13a1	F13a1	1.217863	0.024855	0.568562
Nr1d2	Nr1d2	-1.96682	0.024947	1
Sfi1	Sfi1	2.42208	0.025023	1
Paqr7	Paqr7	-2.84995	0.025082	1
Cilp	Cilp	1.092303	0.025523	0.568562
Akr1e1	Akr1e1	3.384689	0.025574	1
Hephl1	Hephl1	2.581716	0.02563	1
Cmya5	Cmya5	1.211069	0.026063	0.568562
Cma1	Cma1	-1.88069	0.026187	1
Cdsn	Cdsn	-1.15008	0.026806	0.571551

Tsku	Tsku	-3.42553	0.026952	1
Myh7	Myh7	3.971758	0.027014	1
Tgoln2	Tgoln2	-1.23471	0.027106	1
Col24a1	Col24a1	4.556997	0.027867	1
Nhlrc1	Nhlrc1	4.556997	0.027867	1
Fcna	Fcna	2.628556	0.027903	1
Icam1	Icam1	-1.64038	0.028018	1
Samhd1	Samhd1	-1.08289	0.028341	0.583426
Zfp131	Zfp131	-2.21832	0.028416	1
Kctd5	Kctd5	-3.38545	0.028866	1
Cdk14	Cdk14	-1.83338	0.029275	1
Tcea3	Tcea3	3.284257	0.029472	1
Cntn2	Cntn2	4.526251	0.029497	1
Hydin	Hydin	4.526251	0.029497	1
Tmppe	Tmppe	-4.1694	0.029653	1
Ctgf	Ctgf	-1.83646	0.029903	1
Rbm20	Rbm20	4.59375	0.030221	1
Utp23	Utp23	4.59375	0.030221	1
Smpd3	Smpd3	-1.98743	0.03025	1
Trp53inp1	Trp53inp1	-1.89828	0.030315	1
Alkbh1	Alkbh1	3.22294	0.030506	1
Cstf1	Cstf1	2.59779	0.030868	1
Ephx2	Ephx2	2.894185	0.031344	1
AI607873	AI607873	-1.21839	0.031415	0.604747
Spta1	Spta1	3.837058	0.032429	1
Sspo	Sspo	3.82196	0.033066	1
Zfp799	Zfp799	3.822092	0.033167	1
Asb2	Asb2	2.839846	0.033242	1
Cys1	Cys1	-4.12111	0.033348	1
Slfn9	Slfn9	4.495341	0.034601	1
Zxdb	Zxdb	4.495341	0.034601	1
Trmt10c	Trmt10c	3.808102	0.034838	1
Wdr35	Wdr35	3.808141	0.034955	1
Alpk3	Alpk3	2.038776	0.036105	1
Tnnc2	Tnnc2	1.632351	0.036761	0.622832
Htt	Htt	1.396217	0.037036	1
Tbc1d2b	Tbc1d2b	-1.19107	0.037599	0.622832
Ctdspl	Ctdspl	-1.77535	0.038098	1
Prdx6	Prdx6	-1.06182	0.038101	0.622832
Tcf7l1	Tcf7l1	-2.42812	0.03814	1
Ggps1	Ggps1	3.184304	0.038718	1
Rgs5	Rgs5	1.653026	0.038888	1

Gdpd3	Gdpd3	-3.32327	0.039262	1
Fbxo30	Fbxo30	-1.82562	0.039641	1
Snora70	Snora70	-3.3071	0.039848	1
Gm13177	Gm13177	-2.66533	0.039862	1
Cyp2e1	Cyp2e1	-1.68632	0.040206	1
Galnt15	Galnt15	-1.07027	0.040324	0.622832
Gbp7	Gbp7	-1.65182	0.041145	1
Etv3	Etv3	-1.45129	0.041283	1
Tshz1	Tshz1	-2.40036	0.04149	1
Dennd2a	Dennd2a	-1.66301	0.04211	1
Ubl3	Ubl3	-1.41248	0.042457	1
Kat2b	Kat2b	-1.56039	0.042667	1
Nup133	Nup133	2.430995	0.04287	1
Thbs4	Thbs4	1.569851	0.04364	1
Pld4	Pld4	3.13155	0.043899	1
Agpat6	Agpat6	1.413953	0.044267	1
Fa2h	Fa2h	-1.01459	0.044513	0.664364
Pecam1	Pecam1	-1.72964	0.044783	1
Cpne7	Cpne7	4.42578	0.044784	1
Gspt2	Gspt2	4.42578	0.044784	1
Ildr1	Ildr1	4.42578	0.044784	1
Krt83	Krt83	4.42578	0.044784	1
Lrrc39	Lrrc39	4.42578	0.044784	1
Syn1	Syn1	4.42578	0.044784	1
Fbxl15	Fbxl15	4.386558	0.045002	1
Neurl1a	Neurl1a	4.386558	0.045002	1
Pla2g4f	Pla2g4f	4.386558	0.045002	1
Slc38a3	Slc38a3	4.386558	0.045002	1
Cd53	Cd53	-2.75177	0.045188	1
Gcnt2	Gcnt2	-2.37191	0.04522	1
Nrbp2	Nrbp2	2.388124	0.045347	1
Gskip	Gskip	2.647855	0.045352	1
Ubc	Ubc	-1.91332	0.045826	1
Nup37	Nup37	3.70438	0.046102	1
Sema6b	Sema6b	3.70438	0.046102	1
B4galt2	B4galt2	-3.22448	0.046208	1
Gna12	Gna12	-2.251	0.046532	1
Dpep1	Dpep1	-1.01581	0.046821	0.664666
Pcsk7	Pcsk7	2.468747	0.047038	1
Wdr60	Wdr60	2.702734	0.047617	1
Atp6v1b2	Atp6v1b2	-1.45926	0.047619	1
Golga3	Golga3	1.242583	0.047926	1

Tchh	Tchh	2.327368	0.048297	1
Psmb9	Psmb9	-2.56932	0.048438	1
Sos2	Sos2	-2.13253	0.049009	1
Sertad1	Sertad1	-1.63071	0.049113	1
Ifi47	Ifi47	-2.26909	0.049224	1
Senp7	Senp7	2.652941	0.049255	1
Irgm2	Irgm2	-1.71911	0.049343	1
Meg3	Meg3	3.731533	0.049397	1
Wdr37	Wdr37	2.163074	0.049697	1
Serpina3g	Serpina3g	-2.74284	0.049711	1
Hook1	Hook1	2.654264	0.049802	1
Gng2	Gng2	-2.70517	0.049842	1

[0135] *Differences in Extracellular Vesicles RNA-content as an Interaction between age and genotype:*

[0136] Two final sets of differential expression analysis were performed, 3xTg-AD 10 weeks vs. B6129SF2/J 23 weeks and 3xTg-AD 23 weeks vs. B6129SF2/J 10 weeks. These comparisons were made in order to evaluate the effect of both age and genotype on the mRNA expression profile in skin-derived EVs. The 3xTg-AD 10 weeks vs. B6129SF2/J 23 weeks analysis indicated significant upregulation of 67 genes and the downregulation of 89 genes, with no genes being upregulated. Several top pathways that were implicated with these genes include calcium signaling, actin cytoskeleton signaling, and calcium transport I. Analysis of the 3xTg-AD 23 weeks vs. B6129SF2/J 10 weeks comparison revealed the upregulation of 122 genes and downregulation of 134 genes. Subsequent pathway analysis implicated GP6 signaling, corticotropin releasing hormone, and hepatic fibrosis pathways as related to these differentially expressed genes.

[0137] *Murine model 3xtgAD Skin-Derived Extracellular Vesicles Contain Transgenic hAPP/hMAPT mRNA*

[0138] Mutations and aberrant expression of genes related to APP are well documented factors involved in the propagation of neurodegenerative disorders involving amyloidosis such as AD (Shin, J., et al. BMB Rep. 2010 43:704–709; Strang, KH., et al. Lab. Invest. 2019 99:912–928). While mutations in microtubule associated protein Tau (MAPT) are not directly associated with AD (Guo, Q. et al. PLoS One 2013 8:e80706), they have been implicated in frontotemporal dementia (Strang, KH., et al. Lab. Invest. 2019 99:912–928; Guo, Q. et al. PLoS One 2013 8:e80706). More importantly however, these mutations are responsible for the development of tauopathy similar to that seen in AD and can be found in transgenic models of AD (Guo, Q. et al. PLoS One 2013 8:e80706). Here the

potential for skin-derived EVs collected was evaluated from both 3xTg-AD and B6129SF2/J control mice to contain human APP Swedish Mutation (APPswe) and human Tau P301L MAPT transgenes expressed in the 3xTg-AD murine model of Alzheimer's Disease (Oddo, S. et al. Neuron 2003 39:409–421). The presence of these transgenes were characterized through the use of absolute qPCR (n=3) to measure the expression of hAPP and hMAPT mRNA in skin-derived EVs collected at various time points (Figure 5). The resulting gene expression data shows that both hAPP (Figure 5A) and hMAPT (Figure 5B) are expressed significantly in 3xTg-AD skin-derived EVs compared to age matched controls, further indicating that they are capable of harboring potentially neurotoxic genetic cues in relation to the progression of AD within this model.

[0139] *3xTg-AD skin-derived Extracellular Vesicles can transfer neurotoxic hAPP and hMAPT mRNA to neurons in murine primary embryonic neuron cultures*

[0140] Based on results indicating that 3xTg-AD skin-derived EVs are capable of harboring potentially neurotoxic molecular contents, it was decided to proceed and evaluate the neurotoxic capabilities of these EVs directly. While EVs derived from the central nervous system have previously been shown to play a role in neurodegenerative disorders such as Alzheimer's Disease (Sardar Sinha, M. et al. Acta Neuropathol. 2018 136:41–56) and that exosomal proteins are found within amyloid plaques (Rajendran, L. et al. Proc. Natl. Acad. Sci. U. S. A. 2006 103:11172–11177), this pathological potential has yet to be explored in regards to EVs derived from peripheral tissues such as the skin. Therefore, the neurotoxic capabilities of 3xTg-AD and B6129SF2/J skin-derived EVs were examined by exposing 4 different cultures of murine primary neuron cultures to EVs at a concentration of 1-3x10^9 EVs/µL particles for 24 hours followed by RNA isolation for qPCR analysis. The subsequent qPCR results (n=4) show that transgenic hAPP and hMAPT mRNA is significantly expressed in neurons exposed to 3xTg-AD EVs compared to controls (Figure 6A, 6B). These findings indicate that 3xTg-AD skin-derived EVs have the potential to influence the genetic expression profile of neuron targets through the delivery of transgenic mRNA. This observed activity could possibly lead to neurotoxic effects due to expression of APPswe and P301L Tau mutant transgenes, which could in turn advance the onset and/or progression of AD.

[0141] Next the effects of skin-derived EVs on primary neuronal cultures, was evaluated. Equivalent amounts of skin-derived EVs isolated from B612SF2/J and 3xTg-AD at 10 weeks of age. First, EVs from each experimental group were labeled with the PKH26 (Sigma) red fluorescent label and then applied to 8-days primary neuronal cultures for 24h (Figure 7). Qualitative analysis by confocal microscopy shows the presence of fluorescent particles in the cytoplasm of neurons, including both the perinuclear area and the neuronal

projections, identified by Tuj1 immunostaining (Figure 7A, 7B). Furthermore, quantification of cellular uptake shows a higher percentage of neurons uptaking the fluorescent-labeled EVs (Figure 7C). Finally, the neurotoxic effects of EVs derived from the AD mouse model (Figure 8) was studied. 24 hours after exposing primary neuronal cultures to 3xTg-AD or B6129SF2/J skin-derived EVs cell viability was evaluated by epifluorescence microscopy using the Live/Dead kit (Figure 8A). Quantification of neuronal viability shows a cytotoxic effect of EVs in general when compared with unexposed control cultures, however, after 24h there is no difference in cell toxicity between EVs derived from B6129SF2/J or 3xTg-AD mice (Figure 8B).

[0142] Discussion

[0143] The role that peripheral organs and tissues such as the skin and gut play in the onset and progression of neurodegenerative disorders, such as AD and PD, has only recently begun to be understood. While the skin has been suspected to play a role in AD specifically and has even been explored as a possible avenue of diagnosis and drug delivery, no particular mechanism detailing how such a link might be facilitated has been identified. Through the results of the experiments aimed at characterizing the genetic content of skin-derived EVs as AD progresses, changes were identified in the expression of AD related genes such as S100A9 (Figure 4A), as well as expression of APPswe and MAPT P301L mutant genes (Figure 5) responsible for driving the pathology in the 3xTg-AD model of AD.

[0144] Calcium signaling was among the top dysregulated canonical pathways identified by IPA among all the comparison groups. This observation was found in the comparison of differentially expressed genes among the 3xTg-AD 23 week and B6129SF2/J 23 week groups, but absent in the comparison of the 3xTg-AD 10 week and B6129SF2/J 10 week groups. This observation suggests that changes in mRNA expression involved in calcium signaling occur with age in the 3xTg-AD mice that are not seen in B6129SF2/J controls. The dysregulation of calcium signaling has been documented in neurodegenerative diseases such as AD for over a decade and has led to the development of the “calcium hypothesis” of AD (Tong, BCK., et al. Biochim. Biophys. Acta Mol. Cell Res. 2018 1865:1745–1760; Popugaeva, E., et al. Antioxid. Redox Signal. 2018 29:1176–1188). There is growing evidence that the dysregulation of calcium homeostasis in neurons causes synaptic deficits that ultimately lead to the accumulation of amyloid-beta and phosphorylated tau 29 (Popugaeva, E., et al. Antioxid. Redox Signal. 2018 29:1176–1188). In addition to disruptions in calcium signaling, mitochondrial dysfunction, specifically associated with mitochondria-associated ER membrane (MAM), has also been reported in AD (Chakravorty,

A., et al. Front. Aging Neurosci. 2019 11:311). While not identified through IPA as a canonical pathway, two genes involved with calcium signaling within the 3xTg-AD 23 week vs B6129SF2/J 23 week group, ATP2A1 and RYR1, are also associated with MAM function (Schon, EA. & Area-Gomez, E. et al. Mol. Cell. Neurosci. 2013 55:26–36). While MAM associated genes are generally upregulated in AD, the perturbation of MAM function overall is a suspected component of the AD pathology and represents its own hypothesis for how the disease is caused (Area-Gomez, E. & Schon, EA. et al. Curr. Opin. Genet. Dev. 2016 38:90–96).

[0145] Another intriguing find within the 3xTg-AD and B6129SF2/J 23 week group comparisons was the robust upregulation of S100A9 (Log2FoldChange = 6.155). S100A9 is a member of the S100 protein family, which is known to be involved in a multitude of intracellular processes including calcium homeostasis (Cristóvão, JS. & Gomes, CM. SNeurosci. 2019 13:463). As mentioned before, observed differences among the groups in this comparison provide insight into changes in gene expression that occur in skin EVs as AD progression occurs. As such, the finding that S100A9 is heavily upregulated suggests this could be a key change in the genetic profile of skin EVs as AD progresses. Interestingly, this expression pattern is consistent with changes seen in the brain of related TG2576 AD mouse models (Cristóvão, JS. & Gomes, CM. SNeurosci. 2019 13:463). This finding in TG2576 mice is particularly relevant, as this model bears the same APPswe mutations found in the 3xTg-AD used in this study. Furthermore, studies involving the use of S100A9 KO bred with TG2576 mice showed improvements in memory impairment and decreased neuropathology (Kim, H. J. et al. PLoS One 2014 9:e88924). In addition to murine models, upregulation of S100A9 expression has been observed in the brains of AD patients and implicated in AD pathways (Cristóvão, JS. & Gomes, CM. SNeurosci. 2019 13:463). For these reasons, S100A9 has gained a lot of attention as a potential biomarker of AD (Horvath, I. et al. ACS Chem. Neurosci. 2016 7:34–39). Further exploration of the changes and effects of both S100A9 mRNA and protein within skin-derived EVs under AD conditions could prove to be insightful for such purposes. The upregulation of sodium-coupled neutral amino acid transporter 2 (SLC38A2) was also observed within this comparison group. The upregulation of SLC38A2 in 23 week 3xTg-AD skin-derived EVs is consistent with observations of SLC38A2 upregulation with AD progression (Patel, H. et al. Brain Behav. Immun. 2019 80:644–656). The gene for Serpina3b/Serpina3j was found to be upregulated within the 3xTg-AD and B6129SF2/J 23 week group comparisons as well. The complete opposite result was seen when in the B6129SF2/J 23 weeks vs. B6129SF2/J 10 weeks group, where Serpina3b/Serpina3j was found to be downregulated. The fact that this gene is

heavily expressed in aged AD mice while declining with age in controls suggests that its expression is due to the AD condition. This observation of upregulation of Serpina3b/Serpina3j in the skin-derived EVs 3xTg-AD mice is consistent with previous observations of serpina3 upregulation in multiple prion diseases, including AD (Vanni, S. et al. Sci. Rep. 2017 7:15637).

[0146] Absolute qPCR experiments aimed at measuring the expression of transgenic mRNA related to AD in skin-derived EVs further support the notion that they could play a role in AD. The finding that human APPswe and human MAPT P301L mRNA is present in AD skin-derived EVs supports the hypothesis that they are capable of driving the onset and progression of AD. This ultimately suggests the possibility that these EVs are capable of delivering their transgenic mRNA contents to target tissues, which can influence the local protein expression of mutated MAPT and APP within said tissue. This is particularly relevant to AD within the context of the 3xTg-AD model, where the expression of APPswe and MAPT P301L within the CNS is a major component of the pathology.

[0147] In addition, to characterize the effects of mRNAs in AD skin-derived EVs, their neurotoxic potential was evaluated directly in primary neuron EVs exposure experiments. The ability of neurons to uptake mRNA of hAPP and hMAPT from AD skin EVs was demonstrated, suggesting the possibility that aberrant expression of mutated APP and MAPT proteins could possibly occur, which could drive disease progression through subsequent accumulations of beta-amyloid and phosphorylated Tau proteins in the CNS. The results of labeled EVs uptake experiments in primary neurons further support this idea by providing evidence that skin-derived EVs themselves are in fact capable of being uptaken by $Tuj1^+$ cells (Figure 7A, 7B) and do so preferentially over $Tuj1^-$ cells *in vitro* (Figure 7C). Taken together, these observations highlight the potential for skin-derived EVs to interact with neurons and influence expression of AD-related genes within the CNS, ultimately contributing to disease onset and progression. Finally, despite the transfer of potentially neurotoxic cargo from skin-EVs of the 3xTg-AD mice observed here by qRT-PCR and cellular uptake experiments, no increase in neuron toxicity was observed after exposure to 3xTg-AD skin EVs, this may be due to the fact a short-term exposure (24h) was performed (Figure 8), and the cytotoxic effects of APP and Tau may require further incubation times to allow metabolic processing, oligomer formation, and protein accumulation.

[0148] Methods

[0149] *Animal Studies*

[0150] Breeding trios of the triple transgenic mouse model of Alzheimer's Disease (3xTg-AD) B6;129-Tg(APPSwe,TauP301L)1Lfa $Psen1^{tm1Mpm}$/Mmjax (Mutant Mouse

Resource & Research Centers Stock No: 34830-JAX) (Oddo, S. et al. Neuron 2003 39:409–421) and the control strain B6129SF2/J (Stock No: 101045) were purchased from The Jackson Laboratory. Based on recommendations from the donating investigator, only female animals were used for analysis. Mice were housed in groups with *ad libitum* access to water and food in a 12/12 h light/dark cycle (lights on at 6 A.M.) with constant temperature and humidity conditions. All experiments involving animals were performed in accordance with The Ohio State University Institutional Animal Care and Use Committee guidelines (protocol number: 2016A00000074-R1).

[0151] *Skin Extracellular Vesicles Isolation*

[0152] Murine skin-derived EVs were isolated directly from dorsal and ventral 12-mm-diameter skin biopsies. The collected tissue was minced into ~1mm pieces with a surgical scalpel and dissociated using the "37_Multi H" protocol on a "gentleMACS Octo Dissociator" (Miltenyi Biotec Cat. No.130-096-427) in combination with Multi Tissue Dissociation Kit 1 (Miltenyi Biotec #130-110-201). The resulting supernatant was then removed centrifuged at 2000g at 4°C for 30 minutes. The supernatant was then removed and pooled to ensure homogenous EVs concentration and then ½ volume of "Total Exosome Isolation Kit from Cells" (Thermo Fisher Scientific #4478359) was added to it prior to a 12 hour incubation at 4°C. The EVs samples were precipitated with a 10,000g centrifugation at 4 degrees and stored afterwards at -80°C until future use. EVs concentration was measured using a NanoSight Ns3000 (Malvern Pananlytical).

[0153] *Absolute real time PCR of Skin Extracellular-Vesicles*

[0154] Total RNA was extracted from skin EVs pellets using the TRIzol reagent (Thermo Fisher Scientific #15596026) following manufacturer's instructions, followed by measurement of resulting concentration using a NanoDrop 2000 Spectrophotometer (Thermo Fisher #ND-2000). The VILO cDNA synthesis kit (Thermo Fisher Scientific #11756500) was used to perform 20uL reverse transcription reactions. The resulting cDNA was used to measure expression of hAPP and hMAPT using absolute quantification methods with standard curves of plasmids for APP695 (Addgene Plasmid #114193) and TauP301L (Addgene Plasmid #87633) that were generated with a final copy number range of 10^6 copies to 10^2 copies. The aforementioned plasmids were isolated using ZymoPURE II Plasmid Midi Prep Kit (Zymo Research #DG4200). Measurement of isolated plasmid concentration was conducted using a NanoDrop 2000 Spectrophotometer (Thermo Fisher #ND-2000), followed by subsequent calculation of plasmid copy number and dilution of the standard curve. Taqman primers from ThermoFisher were used to amplify genes of interest including: hAPP (Thermo Fisher Scientific #Hs00169098_m1), mAPP (Thermo

Fisher Scientific # Hs00169098_m1), hMAPT (Thermo Fisher Scientific # Hs00902194_m1), and mMAPT (Thermo Fisher Scientific # Mm00521988_m1). All real-time PCR reactions were performed using TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific #44-445-57) and a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific #A28567) using the following cycle settings: 95°C for 10 minutes, followed by 40 cycles of 95°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute.

[0155] *Relative real time PCR of Primary Neurons*

[0156] RNA isolation and cDNA generation were performed using the aforementioned techniques and reagents. Real Time PCR was subsequently performed on primary neurons using the previously mentioned reagents, primers, thermo cycler, and cycle settings. Mouse 18s Ribosomal RNA (Thermo Fisher Scientific #4331182) was used as a housekeeping gene.

[0157] *RNA-seq*

[0158] Total RNA was extracted from extracellular vesicles (EVs) derived from the skin of B6129SF2/J or 3xTg-AD mice using TRIzol™ reagent (ThermoFisher Scientific #15596026) following the manufacturer's directions as previously described. RNA quantification, integrity and quality assessment was obtained with the Qubit Fluorometer (ThermoFisher Scientific #15596026). Libraries were generated with an input of 200ng per sample using the NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina (New England Biolabs #E7760L) and the NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs #E7490) according to the manufacturer's directions. Sequencing was performed on an Illumina Novaseq SP Paired-End 150bp format.

[0159] *RNA-seq Data Analysis*

[0160] The RNA-Seq data was analyzed using Basepair software (https://www.basepairtech.com/) with a pipeline that included the following steps. Reads were aligned to the transcriptome derived from UCSC genome assembly *hg19* using STAR (Dobin, A. et al. Bioinformatics 2013 29:5–21) with default parameters. Read counts for each transcript were measured using featureCounts (Liao, Y., et al. Bioinformatics 2014 30:923–930). Differentially expressed genes were determined using DESeq2 (Love, MI., et al. Genome Biol. 2014 15, 550) and a cut-off of 0.05 on adjusted p-value (corrected for multiple hypotheses testing) was used for creating lists and heatmaps, unless otherwise stated. GSEA was performed on normalized gene expression counts, using gene permutations for calculating p-value.

[0161] *Ingenuity Pathway Analysis (IPA)*

[0162] Pathway analysis of differentially expressed genes was performed using Ingenuity Pathway Analysis software (Qiagen). Core analyses were performed using filtered differential expression data (±.58 log2 fold change and adjusted p-value ≤ .05).

[0163] *Primary Neuronal Cultures*

[0164] Cortical neurons from embryonic day 18.5 C57BL/6J mouse were prepared following the protocol previously reported (Alzate-Correa, D., et al. Methods Mol. Biol. 2020 2050:145–152), including some modifications. The dissected cortices were dissociated with the Neuronal Tissue Dissociation Kit—Postnatal Neurons (Miltenyi Biotec #130-094-802) and incubated for 20 min at 37gentleMACS Octo Dissociator with Heaters (Miltenyi Biotec #130-096-427). Dissociated cells were resuspended in 5% BSA prepared in PBS and Neurons were isolated from the cell suspension using the Neuron Isolation Kit (Miltenyi Biotec #130-115-389). Isolated neurons were resuspended in neuronal culture media composed of Neurobasal™ (Thermo Fisher Scientific #21103049) supplemented with 2 mM GlutaMAX™(Thermo Fisher Scientific #35050061) and 2% NeuroCult™ SM1 Neuronal Supplement (Stemcell Technologies #05711). Neurons were plated on poly-D-lysine (Thermo Fisher Scientific #A3890401) coated glass coverslips (Fisher Scientific #1254580) at a density of $130x10^3$ cell/cm^2. Primary Neuronal Cultures were exposed to skin derived EVs for the specified hours and then processed for Cell viability analysis, fixed for immunocytochemistry or total RNA extraction for qPCR.

[0165] *Cell Viability*

[0166] Cytotoxicity of EVs derived from the skin of B6129SF2/J or 3xTg-AD mice was evaluated with the LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells (Thermo Fisher Scientific #L3224) following manufacturer's instructions. Briefly, after day-in-vitro (DIV) 7 primary neuronal cultures were exposed for 24h to EVs from 10 weeks old B6129SF2/J or 3xTg-AD mice, each well was exposed to 1-$3x10^9$ EVs/µL particles. 24h after exposure neuronal cultures were washed once with sterile PBS followed by incubation at 37°C for 25 minutes with a PBS solution containing calcein AM (1uM) and ethidium homodimer-1 (1um). Finally glass coverslips were fresh mounted using PBS and microphotographs were taken using a Nikon Eclipse 2000 microscope. Cell viability was determined by quantification of the percentage of LIVE (calcein AM positive) and DEAD (ethidium homodimer-1 positive) cells using FIJI ImageJ software (Schindelin, J. et al. Nat. Methods 2012 9:676–682).

[0167] *Labeled Extracellular Vesicles Uptake*

[0168] Pelleted EVs were fluorescently labeled using the "PKH26 Red Fluorescent Cell Linker Kit" (Sigma) following the provided protocol. 8 days after seeding onto coverslips,

primary neuron cultures were exposed to labeled EVs by replacing ⅓ of media volume with media containing 3xTg-AD skin-derived EVs at a concentration of 1-3x109 EVs/µL prior to fixation in 10% formalin 24 hours later. Blocking prior to immunocytochemistry was performed using 5% normal goat serum in PBS-T for 90 minutes. The immunocytochemistry was performed using a Tuj1 antibody (Abcam 107216) in 1:250 PBS-T incubated overnight at 4°C. Afterwards, the samples were washed with PBS-T followed by the addition of secondary antibody (Abcam 150173, λ =488) at a concentration of 1:200 in PBS-T and finally 1:10,000 DAPI in PBS for 1 minute. The coverslips were then mounted onto slides using Vectashield (Vector Labs #H-1700). The slides were subsequently imaged with immunofluorescence and confocal microscopy using a Nikon Eclipse 200 microscope.

[0169] *Statistical Analysis*

[0170] Statistical analyses were conducted using SigmaPlot (version 14) and Graphpad (version 8.4). The qPCR data of skin-derived EVs from 3xTg-AD (3 to 40 weeks) and 3xTg-AD/B6129SF2/J (21 day to 23 weeks) were analyzed using one and two way anovas respectively. Data with adjusted p-values < .05 were considered statistically significant.

Example 3: Evaluating the mRNA content of skin-derived extracellular vesicles for genetic biomarkers of Alzheimer's Disease.

[0171] The purpose of this Example was to identify potential biomarkers of Alzheimer's Disease (AD) through the use of RNA Seq to characterize differences in mRNA expression in skin-derived EVs collected from 3xTg-AD triple transgenic mouse model of AD and B6129SF2/J controls at 10 and 23 weeks of age (n=3). Reads were aligned to the transcriptome derived from UCSC genome assembly *mm10* using STAR (Dobin, A. et al. Bioinformatics 2013 29:15–21) with default parameters. This was followed by differential expression DeSeq2 (Love, M. I., et al. Genome Biol. 2014 15:550) analysis of several different comparison groups of both disease and age including:

3xTg-AD 23 weeks vs. B6129SF2/J 23 weeks (effects of AD in older mice),

3xTg-AD 23 weeks vs. 3xTg-AD 10 weeks (effects of age in AD mice),

3xTg-AD 10 weeks vs. B6129SF2/J 10 weeks (effects of AD in younger mice), and

B6129SF2/J 23 weeks vs. B6129SF2/J 10 weeks (effects of age in control mice).

[0172] The differential expression results were subsequently filtered (p-value ≤ .05, log2foldchange ± 1). Ingenuity Pathway Analysis (IPA, Qiagen) was used to identify overlap between groups, as well as relevant pathways and functions among all groups. In order to identify potential biomarkers, the list of differentially expressed genes in group 1 (effects of AD in older mice) was first examined, showing that any differentially expressed genetic biomarkers would likely be detectable at this point in the 3xTg-AD model, in which cognitive impairment and the first symptoms of AD have been observed at as early as 5 months (~22 weeks) of age (Oddo S., et al. Neuron. 2003 39(3):409-21). While genes in this list alone could contain potential biomarkers, the list was further compared for overlap with group 2 (effects of age in AD mice) to see if any changes in the expression of these genes could be detected between the 10 week and 23 weeks timepoints of AD progression. A list of common genes between these two groups was created, which represents genes that change in expression between weeks 10 and 23 of AD progression that are still detectable at 23 weeks compared with controls. In order to determine how these genes were differentially expressed early on in AD, as well as normal healthy aging, this list of common genes was compared to both group 1 (effects of AD in younger mice) and group 4 (effects of age in control mice) respectively.

[0173] Results

[0174] Differential expression analysis of each group revealed the following differences in gene expression between each group: Group 1 (240 up/426 down), Group 2 (150 up/143 down), Group 3 (91 up/79 down) and Group 4 (98 up/98 down) (Figure 9). For a complete list of differentially expressed genes between each condition, see supplemental document 3. Pathway analysis on all four groups revealed the dysregulation of several pathways that occurred as a result of the AD condition including interleukin-6 signaling (IL6), high mobility group box protein 1 signaling (HMGB1), and interleukin-8 signaling (IL8 (Figure 10A). Examples of some of the top dysregulated disease and function pathways seen in the AD samples include migration of myeloid cells and accumulation of neutrophils (Figure 10B).

[0175] Skin-derived EVs in 3xTg-AD show differential expression of genes and pathways known to be involved in AD that change as the disease progresses. One prominent example of this is S100A8, which has been previously observed to be upregulated in the AD brain (Cristóvão, J. S. & Gomes, C. M. 2019 Front. Neurosci. 13:463). Furthermore, S100A8 upregulation of expression in the brain precedes amyloid plaque formation in TG2576 mice (Lodeiro M, et al. Gerontol A Biol Sci Med Sci. 2017 72(3):319-328) which express the same amyloid precursor protein mutation as the 3xTg-AD model. S100A8 was found to be upregulated in 3xTg-AD skin-derived EVs as a function of age/disease progression, while the opposite was observed in control mice as they age. This indicates that S100A8 could be a potential biomarker found within skin-exosomes of AD given its upregulation is unique to AD and not related to aging. Interestingly however, S100A8 was found to be downregulated in the group 4 comparison (effects of AD in younger mice), which suggests this gene may be down regulated in skin EVs earlier on in disease progression. In addition, a related gene that is also involved in AD, S100A9 (Cristóvão, J. S. & Gomes, C. M. 2019 Front. Neurosci. 13:463), was found to be upregulated in group 1, but not group 2. Another interesting gene found to be upregulated in group 1 was Mitogen-Activated Protein Kinase 13 (MAPK13). MAPK13 has been identified as a major kinase that phosphorylates tau epitopes related to AD neurofibrillary tangles (Cavallini A., et al. J Biol Chem. 2013 288(32):23331-23347). In the group 4 comparison of aged controls vs. young controls, MAPK13 expression showed the opposite trend and was downregulated. Several other differentially expressed genes found in the comparison of group 1 and 2 were also found to be related to AD, such as fatty acid amide hydrolase (D'Addario C., et al. PLoS One. 2012 7(6):e39186) and activity regulated cytoskeleton associated protein (Perez-Cruz., et al. J Neurosci. 2011 31(10):3926-34).

[0176] Pathway analysis on all groups implicated dysregulation of pathways related to known components of AD in group 1, such as calcium signaling (Tong BC, et al. Biochim Biophys Acta Mol Cell Res. 2018 1865(11 Pt B):1745-1760), HMGB1 signaling (Paudel, Y.N., et al. Frontiers in Neuroscience, 2018 12), IL-6 (Cojocaru IM, et al. Rom J Intern Med. 2011 49(1):55-58) signaling, and IL-8 signaling (Ryu, J.K., et al. J Neuroinflammation 2015 12:144) (Figure 10A). The IPA data also

suggested the increase of pathways related to immune and inflammatory responses including: accumulation of neutrophils, which is linked to AD as well as the activities of IL-6 and IL-8 (Park J, et al. Front Immunol. 2019 10:2231) (Figure 10B).

[0177] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Publications cited herein and the materials for which they are cited are specifically incorporated by reference.

[0178] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.