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(54) **METHODS AND COMPOSITIONS FOR SUPPRESSING RETROVIRUSES**

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(57) **ABSTRACT**

The disclosure provides methods and compositions for suppressing retroviruses, including novel methods for treating a retroviral infection in a human in need of such treatment, comprising delivering a functional meiosis arrest female protein 1 (MARF1) to cells containing a retroviral provirus, together with novel expression constructs comprising a coding sequence encoding a functional MARF1 operatively linked to a promoter, vectors comprising such constructs, and packaging cell lines for use in making such vectors.

Specification includes a Sequence Listing.

METHODS AND COMPOSITIONS FOR SUPPRESSING RETROVIRUSES

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application No. 62/260,559, filed Nov. 29, 2015, the contents of which are incorporated herein by reference.

FIELD

[0002] The disclosure relates to the field of gene therapy for treatment and control of retroviral infections, for example HIV infection, and provides methods and compositions therefor.

BACKGROUND

[0003] The Retroviridae are a family of viruses that insert a DNA copy of their genome into the genome of the host cell in order to replicate. A retroviral genome is made up of two single strands of plus-sense RNA. In the retrovirus, the RNA is bound to a capsid protein and encased, along with enzymes necessary for replication, within an envelope made of a retroviral envelope protein in association with glycoproteins and lipids from the membrane of the host cell. The envelope facilitates recognition of the potential host cell and entry into the host cell. Once inside the host cell cytoplasm, the retrovirus releases a reverse transcriptase enzyme to produce DNA from the retroviral RNA genome, which DNA is then incorporated into the host cell genome by a retroviral integrase. The retroviral DNA that is integrated into the host cell genome is called a provirus. The provirus is effectively part of the host cell's genome, translating and transcribing the viral genes along with the cell's own genes, producing the proteins required to assemble new copies of the virus, and transmitting the retroviral DNA to progeny cells.

[0004] If a retrovirus integrates into the germline of its host, it can be passed on for generations. These so-called endogenous retroviruses are believed to make up a significant portion of the human genome.

[0005] About 45% of the human genome is composed of transposable elements. Retrotransposons are a class of these mobile genetic elements that move and replicate in their host through an RNA intermediate using reverse transcriptase. Long terminal repeat (LTR) retrotransposons in particular have close structural resemblance to retroviruses. They are flanked by long terminal direct repeats that contain all of the necessary transcriptional regulatory elements. The autonomous elements (retrotransposons) contain gag and pol genes, which encode a protease, reverse transcriptase, RNAse H and integrase. The main difference between retroviruses and LTR retrotransposons is that retroviruses include a functional envelope gene, so that they can form transmittable viral particles that can infect other cells.

[0006] Retrotranspons may interfere with genome integrity. Mammalian germ cells in particular, have come up with effective tools for retroelement surveillance. In mammals, transposon surveillance is crucial in oocytes during fetal oocyte attrition, a process where about 75% of egg cells are deemed "inadequate" and die. In oocytes, MARF1 functions as a retrotransposon surveillance tool. Mutations of MARF1 cause female infertility.

[0007] Human immunodeficiency virus (HIV) is a retrovirus that causes Acquired Immunodeficiency Syndrome (AIDS) in humans. Since 1981, an estimated 25 million people are have died from HIV infections globally. The disease has proven extremely difficult to treat. The viral envelope is made up largely of human glycoproteins and lipids, while the viral envelope protein—the only viral DNA exposed to the immune system—is variable, and the virus may be dormant and virtually undetectable for long periods. This makes the development of effective vaccines challenging.

[0008] HIV can be divided into two major types, HIV type 1 (HIV-1) and HIV type 2 (HIV-2). The HIV-1 Group M viruses predominate and are responsible for the AIDS pandemic. HIV-1 Group M can be further subdivided into subtypes based on genetic sequence data. Some of the subtypes are known to be more virulent or are resistant to different medications. HIV-2 viruses are thought to be less virulent and transmissible than HIV-1 Group M viruses, although HIV-2 is also known to cause AIDS. While HIV-1 Group M subtypes C and A make up about 74% of HIV infections globally, most infections in North America and Europe are subtype B.

[0009] Glycoproteins in the HIV envelope bind to CD4+ T helper cells, as well as macrophages and dendritic cells. Once bound, the retrovirus enters the cell by membrane fusion and starts transcribing its RNA genome to a DNA genome on its way to the nucleus. The HIV includes integrase, which integrates this DNA into the host genome as a provirus. When activated, the provirus transcribes mRNA, as well as enzymes and structural proteins to make up the virus. The viral mRNA is transported out to the cytoplasm where packaging occurs. The packaged virions then bud out of the host cell, carrying with them lipids and glycoproteins from the host cell membrane, to infect more cells. The HIV provirus may be relatively dormant for long periods, and is able to hide from the immune system by producing undetectable amounts of viral proteins. An unknown signal causes the HIV provirus to "activate," so that it replicates and releases virus. The infected cells may be killed by the virus directly or via triggering of apoptosis, or they may be destroyed by cytotoxic CD8+ cells that recognize a foreign antigen on the surface of the infected cells. Once the infected cell types, particularly the CD4+ T cells, decline below a critical level, the immune system can no linger provide cell-mediated immunity, and the patient becomes very susceptible to opportunistic infections and cancers.

[0010] An HIV positive person is diagnosed with AIDS when his or her CD4 cell count falls below 200 cells/μl or CD4 T-lymphocyte percentage of total lymphocytes is less than 14%.

[0011] HIV has a unique pseudodiploid genome, as well as low replication fidelity, and a complex viral envelope, making it able to evolve quickly and develop resistance to drugs or vaccines. The viral proliferation can be inhibited by aggressive antiviral therapies such as HAART (Highly Active Antiretroviral Therapy), which is a triple (or more)—drug therapy method combining inhibitors of binding and entry, reverse transcriptase, integration, transcription, assembly, and release. Such combinations therapies are very expensive and debilitating. There is, however, no cure for the disease, so the therapy must be continued for as long as the patient can tolerate the side effects or until the virus evolves to become resistant to the treatment.

[0012] New and better alternatives to treatment of retroviral infections are urgently needed.

SUMMARY

[0013] This invention provides a new approach to treatment of retroviral infections, by using MARF1 to silence the retroviral provirus.

[0014] The invention provides in some embodiments, methods of treating a retroviral infection in a human in need of such treatment, comprising delivering a functional meiosis arrest female protein 1 (MARF1) to cells containing a retroviral provirus, for example by means of a DNA vector such as an adenoviral (AV) vector. In other embodiments, the invention provides vectors comprising sequences encoding MARF1 and packaging cell lines that express such vectors.

[0015] Further areas of applicability of the present invention will become apparent from the detailed description provided hereinafter. It should be understood that the detailed description and specific examples, while indicating the preferred embodiment of the invention, are intended for purposes of illustration only and are not intended to limit the scope of the invention.

DETAILED DESCRIPTION

[0016] The invention this provides, in one embodiment, a method (Method 1) of treating a retroviral infection in a human in need of such treatment, comprising delivering a functional meiosis arrest female protein 1 (MARF1) to cells containing a retroviral provirus. By functional MARF1 is meant a human MARF1 protein or a fragment or variant thereof which retains MARF1 activity in human cells, e.g., which is capable of repressing transposable elements and inhibiting their mobilization.

[0017] For example, the invention provides

[0018] 1.1.Method 1, wherein the MARF1 is delivered by means of a DNA vector comprising an expression cassette having a coding sequence encoding a functional MARF1 operatively linked to a promoter, that which will express MARF1 in said cells containing a retroviral provirus.

[0019] 1.2.Method 1.1 wherein the DNA vector is a replication-deficient viral vector.

[0020] 1.3.Method 1.2 wherein the replication-deficient viral vector is an adenoviral (AV) vector.

[0021] 1.4.Method 1.3 wherein the AV vector is not capable of integration into the genome of said cells containing a retroviral provirus.

[0022] 1.5.Method 1.2 wherein the replication-deficient viral vector is an adeno-associated viral (AAV) vector.

[0023] 1.6.Method 1.5 wherein the AAV vector is not capable of integration into the genome of said cells containing a retroviral provirus.

[0024] 1.7.Any of foregoing Methods 1.1 to 1.6 wherein the DNA vector transiently expresses MARF1 in the cells containing a retroviral provirus but does not integrate into the genome of said cells.

[0025] 1.8.Any of Methods 1.1, 1.2 or 1.3 wherein the cells containing a retroviral provirus are permanently transformed with a gene constitutively expressing MARF1.

[0026] 1.9.Any foregoing method wherein the gene expressing a functional MARF1 comprises a cDNA encoding a human MARF1.

[0027] 1.10. Any foregoing method wherein the MARF1 is a human MARF1.

[0028] 1.11. Any foregoing method wherein the MARF1 has at least 90% amino acid sequence identity, e.g. at least 95% identity, e.g., at least 99% identity, e.g., as measured by a BLAST algorithm, to a MARF1 selected from GenBank Accession: NP_055462.2, NP_001171927.1 and NP_001171928.1.

[0029] 1.12. Any foregoing method wherein the MARF1 has at least 90% amino acid sequence identity, e.g. at least 95% identity, e.g., at least 99% identity, e.g., as measured by a BLAST algorithm, to SEQ ID: 1

SEQ ID 1:

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   1  mmegngtens csrtrgwlqq dndakpwlwk fsncfsrpeq tlphspqtke ymenkkvave

  61  lkdvpsplha gsklfpavpl pdirslqqpk iqlssvpkvs ccahcpneps tspmrfgggg

 121  ggsggtssli hpgalldsqs trtitcqvgs gfafqsassl qnasarnnla giasdfpsmc

 181  lesnlssckh lpccgklhfq schgnvhklh qfpslqgcts agyfpcsdft sgapghleeh

 241  isqseltphl ctnslhlnvv ppvclkgsly cedclnkpar nsiidaakvw pnipppntqp

 301  aplavplcng cgtkgtgket tlllatslgk aaskfgspev avagqvlenl ppigvfwdie

 361  ncsvpsgrsa tavvqrirek ffkghreaef icvcdisken keviqelnnc qvtvahinat

 421  aknaaddklr qslrrfanth tapatvvlvs tdvnfalels dlrhrhgfhi ilvhknqase

 481  allhhaneli rfeefisdlp prlplkmpqc htllyvynlp ankdgksysn rlrrlsdncg

 541  gkvlsitgcs ailrfinqds aeraqkrmen edvfgnriiv sftpknrelc etkssnaiad

 601  kvkspkklkn pklclikdas eqsssakatp gkgsqansgs atkntrwksl qelcrmeskt

 661  ghrnsehqqg hlrlvvpthg nssaaystpk nsgvaepvyk tsqkkenlsa rsvtsspvek

 721  kdkeetvfqv sypsafsklv asrqvsplla sqswssrsms pnllnraspl afnianssse

 781  adcpdpfang advqvsnidy rlsrkelqql lqeafarhgk vksvelspht dyqlkavvqm

 841  enlqdaigav nslhrykigs kkilvslatg aaskslslls aetmsvlqda pacclplfkf
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 901   tdiyekkfgh klnvsdlykl tdtvaireqg ngrlycllps sqarqsplgs sqshdgsstn

 961   cspiifeele yhepvcrqhc snkdfsehef dpdsykipfv ilslktfapq vhsllqtheg

1021   tvpllsfpdc yiaefgdlev vqenqggvpl ehfitcvpgv niataqngik vvkwihnkpp

1081   ppntdpwllr skspvgnpql iqfsrevidl lksqpscvip ishfipsyhh hfakqcrvsd

1141   ygyskliell eavphylqil gmgskrlltl thraqvkrft qdllkllksq askqvivref

1201   sqayhwcfsk dwdvteygvc elidivseip dtticlsqqd nemvicipkr ertqdeiert

1261   kqfskdvvdl lrhqphfrmp fnkfipsyhh hfgrqcklay ygftkllelf eaipdtlqvl

1321   ecgeekiltl teverfkala aqfvkllrsq kdnclmmtdl lteyaktfgy tfrlqdydvs

1381   sisaltqklc hvvkvadies grqiqlinrk slrsltaqll vllmswegtt hlsveelkrh

1441   yesthntpin pceygfmtlt ellkslpylv evftndkmee cvkltslylf aknvrsllht

1501   yhyqqiflhe fsmaytkyvg etlqpktygh ssveellgai pqvvwikghg hkrivvlknd

1561   mksrlsslsl spanhenqps egerilevpe shtaselklg adgsgpshte qellrltdds

1621   pvdllcapvp sclpspqlrp dpvilqsadl iqfeerpqep seimilnqee kmeipipgks

1681   ktltsdssss cisaavpvpp cpssetsesl lskdpvespa kkqpknrvkl aanfslapit

1741   kl
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[0030] 1.13. Any foregoing method wherein the MARF1 comprises one or more, or 5 or more, or all of the following conserved sequences from the sequence of SEQ ID NO 1: 352-494, 1487-1562, 1004-1074, 1100-1171, 1260-1330, 1176-1247, 1412-1484, 787-875, 510-582, 1336-1406, 876-937, 795-865.

[0031] 1.14. Any foregoing method wherein the MARF1 comprises residues 352-1562 of SEQ ID NO 1.

[0032] 1.15. Any foregoing Method 1.1, et seq. wherein the sequence encoding a functional MARF1 is a sequence which encodes a protein which comprises one or more, or 5 or more, or all of the following conserved sequences from the sequence of SEQ ID NO 1: 352-494, 1487-1562, 1004-1074, 1100-1171, 1260-1330, 1176-1247, 1412-1484, 787-875, 510-582, 1336-1406, 876-937, 795-865; e.g., a protein which comprises residues 352-1562 of SEQ ID 1.

[0033] 1.16. Any foregoing Method 1.1, et seq. wherein the sequence encoding a functional MARF1 is a sequence which has at least 90% sequence identity, e.g. at least 95% identity, e.g., at least 99% identity, e.g., as measured by a BLAST algorithm, to the coding sequence (CDS) of a nucleotide sequence selected from GenBank Accession: NM_014647.3, NM_001184998.1, and NM_001184999.1.

[0034] 1.17. Any foregoing Method 1.1 et seq. wherein the promoter is a viral promoter.

[0035] 1.18. Any foregoing Method 1.1 et. seq. wherein the promoter is selected from cytomegalovirus (CMV) and chicken β-actin (CBA) promoters.

[0036] 1.19. Any foregoing Method wherein the retroviral infection is a lentiviral infection.

[0037] 1.20. Any foregoing Method wherein the retroviral infection is human immunodeficiency virus (HIV), e.g., HIV-1 or HIV-2, e.g., HIV-1, e.g. HIV-1 Group M, one or more of HIV-1 Group M subtypes A, B or C.

[0038] 1.21. Any foregoing method wherein the cells containing a retroviral provirus are white blood cells.

[0039] 1.22. Any foregoing method wherein the cells containing a retroviral provirus are CD4+ lymphocytes.

[0040] 1.23. Any foregoing Method 1.1 et seq. wherein the functional meiosis arrest female protein 1 (MARF1) is delivered to the cells containing a retroviral provirus

[0041] 1.23.1. In vivo, e.g., by injection; or

[0042] 1.23.2. Ex vivo, e.g., by removing from the patient's body, treating, and returning the cells containing a retroviral provirus.

[0043] 1.24. Any foregoing a method comprising

[0044] taking blood from the patient, and optionally further isolating cells containing a retroviral provirus from the blood, e.g. CD4+ cells;

[0045] delivering the functional meiosis arrest female protein 1 (MARF1) to the cells containing a retroviral provirus;

[0046] and returning said blood or cells to the patent's circulatory system.

[0047] 1.25. Any foregoing method wherein the patient receives antiviral medications which will inhibit retroviral expression but will not inhibit AAV vector delivery and expression, e.g., medications selected from one or more of

[0048] 1.25.1. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

[0049] 1.25.2. Nucleoside reverse transcriptase inhibitors (NRTIs)

[0050] 1.25.3. Protease inhibitors (PIs)

[0051] 1.25.4. Fusion inhibitors

[0052] 1.25.5. CCRS antagonists (CCR5s)

[0053] 1.25.6. Integrase strand transfer inhibitors (INSTIs)

[0054] 1.26. Any foregoing method wherein subsequent to treatment with MARF1, the patient receives antiviral medications to inhibit reinfection by the retrovirus, e.g., medications selected from one or more of

[0055] 1.26.1. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

[0056] 1.26.2. Nucleoside reverse transcriptase inhibitors (NRTIs)

[0057] 1.26.3. Protease inhibitors (PIs)

[0058] 1.26.4. Fusion inhibitors

[0059] 1.26.5. CCRS antagonists (CCR5s)

[0060] 1.26.6. Integrase strand transfer inhibitors (INSTIs)

[0061] 1.27. Any foregoing method comprising transforming cells containing a retroviral provirus using a vector according to any if Vector 1, et seq. below.

[0062] In another embodiment, the invention provides a DNA vector (Vector 1) comprising an expression cassette having a coding sequence encoding a functional MARF1 operatively linked to a promoter, e.g., a heterologous promoter, e.g. wherein the expression cassette can express said coding sequence to provide MARF1 in cells containing a retroviral provirus.

[0063] 1.1. Vector 1 wherein the DNA vector is a replication-deficient viral vector.

[0064] 1.2. Vector 1.1 wherein the replication-deficient viral vector is an adenoviral (AV) vector.

[0065] 1.3. Vector 1.3 wherein the AV vector is not capable of integration into the genome of said cells containing a retroviral provirus.

[0066] 1.4. Vector 1.1 wherein the replication-deficient viral vector is an adeno-associated viral (AAV) vector, e.g. AAV2, 4, 5, 8, or 9, or a synthetic AAV vector such as AAV-DJ (synthetic serotype made from DNA family shuffling of 8 wild type serotypes of AAV, including AAV2, 4, 5, 8, 9, avian, bovine and goat AAV) or self complementary adeno-associated virus (scAAV).

[0067] 1.5. Vector 1.4 wherein the AAV vector is not capable of integration into the genome of said cells containing a retroviral provirus.

[0068] 1.6. Any foregoing Vector wherein the vector is incapable of integrating to the genome of the cells containing a retroviral provirus but will transiently express MARF1 in said cells, e.g., wherein the vector lacks functional elements necessary for integration, e.g., lacks functional rep and/or cap elements.

[0069] 1.7. Any of Vector 1, 1.1, 1.2 or 1.4 wherein the vector is capable of permanently transforming cells containing a retroviral provirus with a gene constitutively expressing a functional MARF1.

[0070] 1.8. Any foregoing Vector wherein the MARF1 is a human MARF1.

[0071] 1.9. Any foregoing Vector wherein the MARF1 has at least 90% amino acid sequence identity, e.g. at least 95% identity, e.g., at least 99% identity, e.g., as measured by a BLAST algorithm, to a MARF1 selected from GenBank Accession: NP_055462.2, NP_001171927.1 and NP_001171928.1.

[0072] 1.10. Any foregoing Vector wherein the functional MARF1 has at least 90% amino acid sequence identity, e.g. at least 95% identity, e.g., at least 99% identity, e.g., as measured by a BLAST algorithm, to SEQ ID: 1.

[0073] 1.11. Any foregoing Vector wherein the functional MARF1 comprises one or more, or 5 or more, or all of the following conserved sequences from the sequence of SEQ ID 1: 352-494, 1487-1562, 1004-1074, 1100-1171, 1260-1330, 1176-1247, 1412-1484, 787-875, 510-582, 1336-1406, 876-937, 795-865.

[0074] 1.12. Any foregoing Vector wherein the functional MARF1 comprises residues 352-1562 of SEQ ID 1.

[0075] 1.13. Any foregoing Vector wherein the sequence encoding a functional MARF1 is a sequence which encodes a protein which comprises one or more, or 5 or more, or all of the following conserved sequences from the sequence of SEQ ID 1: 352-494, 1487-1562, 1004-1074, 1100-1171, 1260-1330, 1176-1247, 1412-1484, 787-875, 510-582, 1336-1406, 876-937, 795-865; e.g., a protein which comprises residues 352-1562 of SEQ ID 1.

[0076] 1.14. Any foregoing Vector wherein the sequence encoding a functional MARF1 is a sequence which has at least 90% sequence identity, e.g. at least 95% identity, e.g., at least 99% identity, e.g., as measured by a BLAST algorithm, to the coding sequence (CDS) of a nucleotide sequence selected from GenBank Accession: NM_014647.3, NM_001184998.1, and NM_001184999.1

[0077] 1.15. Any foregoing Vector wherein the promoter is a heterologous promoter, e.g. a promoter different from a natural MARF1 promoter, e.g. wherein the coding sequence encoding a functional MARF1 and the promoter are derived from different species.

[0078] 1.16. Any foregoing Vector wherein the promoter is a constitutive promoter.

[0079] 1.17. Any foregoing Vector wherein the promoter is a viral promoter.

[0080] 1.18. Any foregoing Vector wherein the promoter is selected from cytomegalovirus (CMV) promoter, chicken β-actin (CBA) promoter, and CAG promoter (i.e., hybrid of the cytomegalovirus (CMV) early enhancer element and chicken beta-actin promoter).

[0081] 1.19. Any foregoing Vector further comprising a selectable marker, e.g., an antibiotic resistance gene.

[0082] In other embodiments, the invention provides a packaging cell line which expresses a vector as described in Vector 1 et seq. For example, where the vector is a replication-deficient viral vector, the packaging cell line will express in trans the genes required to permit replication of the vector in the cell line. For example, in some embodiments, to introduce high levels of expressed MARF1 in somatic cells, the vector may be an adenoviral (AV) vector, e.g., a serotype 5 adenovirus, wherein the adenovirus is engineered to be nonreplicative and nonpathogenic, e.g., by deletion of all or part of E1 (which is necessary for replication) and E3 (to provide room for insert of the gene of interest). In this case, the vector can be produced in a packaging cell line that is engineered to express the E1 necessary for the virus to replicate. AV vectors are useful because they infect and express in many cell types including nondividing cells and typically will not integrate into the human genome. In other embodiments, AAV vectors may be used, provided the size of the MARF1 expression cassette is small enough to fit in the AAV vector, e.g., less than about 4.7 Kb. In this embodiment, the recombinant AAV will preferably have the replication and capsid genes are provided in trans (in pRep/Cap plasmid), so that only the two ITRs of AAV genome are left and packaged into virion, while the adenovirus genes required are provided either provided by adenovirus or another plasmid, so that risk for the recombinant AAV to replicate in the cells is very low. For example, in one embodiment, the AAV vector is made by co-transfection of AAV production cells with three plasmids: (1) an AAV2 ITR-containing plasmid carrying the MARF1 expression cassette; (2) a plasmid that carries the AAV2 Rep-Cap; and (3) a plasmid that provides the helper genes

isolated from adenovirus. In some embodiments, to avoid risk of triggering an immune response to the vector, the vector is administered ex vivo, to blood cells, for example CD4+ cells, which are removed, transfected, and then returned to the body only after the vector titer is substantially reduced. In some embodiments, the vector will comprise a selectable marker, for example an antibiotic resistance gene, wherein the antibiotic is one such as kanamycin which would kill the packaging cell line in the absence of the resistance gene, so that the packaging cell line is under selective pressure to produce the vector. Non-replicating AV and AAV vectors are commercially available from many sources, e.g., from Vector Biolabs (Malvern, Pa.) or Agilent (Santa Clara, Calif.), for example Agilent's AdEasy adenoviral vector system that uses recombination with a phage produced in *E. coli* to insert the gene of interest.

[0083] In some embodiments the invention provides MARF1 or a vector comprising a gene for MARF1, e.g., as described in Vector 1, et seq., as a therapeutic agent, e.g., for treatment of a retroviral infection in a human, e.g., in accordance with Method 1 et seq. The invention further provides the use of a sequence expressing MARF1 in the manufacture of a therapeutic agent, e.g., Vector 1 et seq., e.g., for use in treating a retroviral infection in a human, e.g., in accordance with Method 1 et seq.

[0084] Without intending to be bound by theory, it is proposed that the mechanism of MARF1's efficacy to silence a retroviral provirus is similar to the mechanism as seen in MARF1's silencing of retrotransposons in oocytes.

[0085] Certain types of transposable elements, for example Class 1 transposable elements or retrotransposons, are first transcribed from DNA to RNA, then the RNA produced is reverse transcribed to DNA. This copied DNA is then inserted at a new position into the genome. The reverse transcription step is catalyzed by a reverse transcriptase, which may be encoded by a coding region in the transposable element. Retroviruses like HIV and some retrotransposons both contain long terminal repeats (LTRs) and encode reverse transcriptase. Indeed, it is thought that retrotransposons may be descended from ancient retroviral infections. The LTRs act to mediate integration of the retroviral DNA via an LTR specific integrase into the host chromosome. A retroviral provirus can thus be understood as a particular type of eukaryotic retrotransposon, which can produce RNA intermediates that, rather than producing DNA which reintegrates into the host genome, will leave the host cell and produce DNA that can integrate into the genome of other cells.

[0086] While the exact mechanism is not known, it appears that MARF1 recognizes and stimulates methylation of retrotransposons in oocytes. Once the retrotransposons are methylated, their expression is also suppressed in progeny cells. Thus MARF1 is not normally expressed or needed in somatic cells, because the gene silencing of the retrotransposons is already carried out in the oocyte and persists in the somatic cells. But somatic cells are capable of suppressing expression of genes through methylation or other epigenetic means, so the basic mechanism for suppression normally should be available in somatic cells as in oocytes.

[0087] While the regulators for gene expression may vary (primarily MARF1 in oocytes, primarily tumor suppressor factors in somatic cells) the basic machinery for gene suppression (e.g., histone deacetylase and DNA methylase) appears to be ubiquitous, and thus potentially available for use by MARF1 if MARF1 is provided to somatic cells.

[0088] Azidothymidine (AZT) is a nucleoside analog reverse-transcriptase inhibitor that was used as an effective antiviral for HIV until a mutant AZT-resistant strain took over. AZT is shown to have a profound effect on fetal oocyte attrition, increasing oocyte numbers in mice, as FOA is largely dependent on favoring oocytes with less LINE-1 retrotransposon activity. This suggests some functional homology between retroviral proviruses and retrotransposons and supports the theory that a mechanism that silences one could be effective to silence the other.

[0089] One could think of a cell infected with a retrovirus as facing a problem (a potentially disruptive retrotransposon), which is routinely managed with extremely high efficiency at the oocyte level, but which is only rarely seen at the somatic cell level. The proposal involves bringing a tool from the oocyte (MARF1) to fix an analogous problem in a somatic cell.

[0090] In some embodiments, the invention involves administering to HIV positive patients a vector expressing the meiosis arrest female 1 (MARF1) gene, or alternatively, isolating, treating and reintroducing their CD4+ T-cells. The MARF1 should permanently suppress the HIV provirus by selective methylation, so that the provirus will also be suppressed in progeny cells. CD4+ T-cells with HIV provirus suppressed should have a selective advantage over the non-suppressed cells.

[0091] Although the expression of the retroviral provirus in a particular cell line will be permanently silenced following exposure to MARF1, there is still some risk of reinfection from cells that are not treated with the MARF1. This risk can be reduced by continued treatment with conventional antiviral therapies, which suppress infection, or it may be desirable to use a vector that will permanently transform cells containing a retroviral provirus with a gene constitutively expressing MARF1, so that such cells will be resistant to subsequent retroviral infection. This approach has the advantage that the transformed cells will have a selective advantage over the nontransformed cells in the presence of the virus, but may also carry additional risks, however, insofar as the integration of the MARF1 transgene may disrupt other genes, and the long-term effect of constitutive MARF1 expression is not known.

[0092] The foregoing description of certain preferred embodiment(s) and the following examples are in no way intended to limit the invention, its application, or uses.

EXAMPLE 1

Demonstration that MARF1 can Silence Genes in Somatic Cells (CD4+ Cells)

[0093] MARF1 should be able to target first a variety of retrotransposons, and then a retrovirus, as a retrovirus is essentially a retrotranspon with envelope protein. Introducing MARF1 into somatic cells should show a decrease in levels of retrotransposon mRNA and when tested with a retrovirus, show a decrease in virus titer.

[0094] CD4+ cells (SUP-T1, although Jurkat cells expressing CD4 may be used instead) are transformed using adenovirus/retrotransposon hybrid vectors describe by Kubo et al. to insert a L1RP retrotransposon/GFP indicator transgene. See Kubo, S., & Soifer, H. "779. *High-Capacity Adenovirus/Retrotransposon Hybrid Vectors for Efficient and Stable Gene Transfer*". Molecular Therapy, (2004) 9: S295. AV-MARF1 vector is prepared using Adeno-X Expression System 3 (Clontech) and MARF1 cDNA corresponding to the coding sequence (CDS) of GenBank Accession: NM_014647.3.

[0095] GFP expression is measured in the transformed cells in combination with (i) AV-MARF1 vector or (ii) AV-blank vector (control). Transfection with AV-MARF1 suppresses GFP expression in the cells.

EXAMPLE 2

Efficacy of MARF1-AV Vector in HIV-Infected Cell Line

[0096] A CD4+ cell line highly susceptible to HIV infection is created, e.g., generally as described in Krowicka H, Robinson J E, Clark R, Hager S, Broyles S, Pincus S H. Use of Tissue Culture Cell Lines to Evaluate HIV Antiviral Resistance. *AIDS Research and Human Retroviruses.* 2008; 24(7):957-967. The cells are infected with HIV and then one group is treated with MARF1-AV, the other with a blank AV vector. Levels of HIV Gag polyprotein are monitored at different points after infection. Decrease in polyprotein level will correlate with inhibition of replication. Gag protein levels can be determined using a radioimmunoassay (RIA).

SEQUENCE LISTING

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<211> LENGTH: 1742
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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Trp Leu Gln Gln Asp Asn Asp Ala Lys Pro Trp Leu Trp Lys Phe Ser
            20                  25                  30

Asn Cys Phe Ser Arg Pro Glu Gln Thr Leu Pro His Ser Pro Gln Thr
        35                  40                  45

Lys Glu Tyr Met Glu Asn Lys Lys Val Ala Val Glu Leu Lys Asp Val
    50                  55                  60

Pro Ser Pro Leu His Ala Gly Ser Lys Leu Phe Pro Ala Val Pro Leu
65                  70                  75                  80

Pro Asp Ile Arg Ser Leu Gln Gln Pro Lys Ile Gln Leu Ser Ser Val
                85                  90                  95

Pro Lys Val Ser Cys Cys Ala His Cys Pro Asn Glu Pro Ser Thr Ser
            100                 105                 110

Pro Met Arg Phe Gly Gly Gly Gly Gly Gly Ser Gly Gly Thr Ser Ser
        115                 120                 125

Leu Ile His Pro Gly Ala Leu Leu Asp Ser Gln Ser Thr Arg Thr Ile
    130                 135                 140

Thr Cys Gln Val Gly Ser Gly Phe Ala Phe Gln Ser Ala Ser Ser Leu
145                 150                 155                 160

Gln Asn Ala Ser Ala Arg Asn Asn Leu Ala Gly Ile Ala Ser Asp Phe
                165                 170                 175

Pro Ser Met Cys Leu Glu Ser Asn Leu Ser Ser Cys Lys His Leu Pro
            180                 185                 190

Cys Cys Gly Lys Leu His Phe Gln Ser Cys His Gly Asn Val His Lys
        195                 200                 205

Leu His Gln Phe Pro Ser Leu Gln Gly Cys Thr Ser Ala Gly Tyr Phe
    210                 215                 220

Pro Cys Ser Asp Phe Thr Ser Gly Ala Pro Gly His Leu Glu Glu His
225                 230                 235                 240

Ile Ser Gln Ser Glu Leu Thr Pro His Leu Cys Thr Asn Ser Leu His
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               245                 250                 255

Leu Asn Val Val Pro Pro Val Cys Leu Lys Gly Ser Leu Tyr Cys Glu
            260                 265                 270

Asp Cys Leu Asn Lys Pro Ala Arg Asn Ser Ile Ile Asp Ala Ala Lys
        275                 280                 285

Val Trp Pro Asn Ile Pro Pro Pro Asn Thr Gln Pro Ala Pro Leu Ala
    290                 295                 300

Val Pro Leu Cys Asn Gly Cys Gly Thr Lys Gly Thr Gly Lys Glu Thr
305                 310                 315                 320

Thr Leu Leu Leu Ala Thr Ser Leu Gly Lys Ala Ala Ser Lys Phe Gly
                325                 330                 335

Ser Pro Glu Val Ala Val Ala Gly Gln Val Leu Glu Asn Leu Pro Pro
            340                 345                 350

Ile Gly Val Phe Trp Asp Ile Glu Asn Cys Ser Val Pro Ser Gly Arg
        355                 360                 365

Ser Ala Thr Ala Val Val Gln Arg Ile Arg Glu Lys Phe Phe Lys Gly
    370                 375                 380

His Arg Glu Ala Glu Phe Ile Cys Val Cys Asp Ile Ser Lys Glu Asn
385                 390                 395                 400

Lys Glu Val Ile Gln Glu Leu Asn Asn Cys Gln Val Thr Val Ala His
                405                 410                 415

Ile Asn Ala Thr Ala Lys Asn Ala Ala Asp Asp Lys Leu Arg Gln Ser
            420                 425                 430

Leu Arg Arg Phe Ala Asn Thr His Thr Ala Pro Ala Thr Val Val Leu
        435                 440                 445

Val Ser Thr Asp Val Asn Phe Ala Leu Glu Leu Ser Asp Leu Arg His
    450                 455                 460

Arg His Gly Phe His Ile Ile Leu Val His Lys Asn Gln Ala Ser Glu
465                 470                 475                 480

Ala Leu Leu His His Ala Asn Glu Leu Ile Arg Phe Glu Glu Phe Ile
                485                 490                 495

Ser Asp Leu Pro Pro Arg Leu Pro Leu Lys Met Pro Gln Cys His Thr
            500                 505                 510

Leu Leu Tyr Val Tyr Asn Leu Pro Ala Asn Lys Asp Gly Lys Ser Val
        515                 520                 525

Ser Asn Arg Leu Arg Arg Leu Ser Asp Asn Cys Gly Gly Lys Val Leu
    530                 535                 540

Ser Ile Thr Gly Cys Ser Ala Ile Leu Arg Phe Ile Asn Gln Asp Ser
545                 550                 555                 560

Ala Glu Arg Ala Gln Lys Arg Met Glu Asn Glu Asp Val Phe Gly Asn
                565                 570                 575

Arg Ile Ile Val Ser Phe Thr Pro Lys Asn Arg Glu Leu Cys Glu Thr
            580                 585                 590

Lys Ser Ser Asn Ala Ile Ala Asp Lys Val Lys Ser Pro Lys Lys Leu
        595                 600                 605

Lys Asn Pro Lys Leu Cys Leu Ile Lys Asp Ala Ser Glu Gln Ser Ser
    610                 615                 620

Ser Ala Lys Ala Thr Pro Gly Lys Gly Ser Gln Ala Asn Ser Gly Ser
625                 630                 635                 640

Ala Thr Lys Asn Thr Asn Val Lys Ser Leu Gln Glu Leu Cys Arg Met
                645                 650                 655
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Glu Ser Lys Thr Gly His Arg Asn Ser Glu His Gln Gln Gly His Leu
            660                 665                 670

Arg Leu Val Val Pro Thr His Gly Asn Ser Ser Ala Ala Val Ser Thr
        675                 680                 685

Pro Lys Asn Ser Gly Val Ala Glu Pro Val Tyr Lys Thr Ser Gln Lys
    690                 695                 700

Lys Glu Asn Leu Ser Ala Arg Ser Val Thr Ser Ser Pro Val Glu Lys
705                 710                 715                 720

Lys Asp Lys Glu Glu Thr Val Phe Gln Val Ser Tyr Pro Ser Ala Phe
                725                 730                 735

Ser Lys Leu Val Ala Ser Arg Gln Val Ser Pro Leu Leu Ala Ser Gln
            740                 745                 750

Ser Trp Ser Ser Arg Ser Met Ser Pro Asn Leu Leu Asn Arg Ala Ser
        755                 760                 765

Pro Leu Ala Phe Asn Ile Ala Asn Ser Ser Ser Glu Ala Asp Cys Pro
    770                 775                 780

Asp Pro Phe Ala Asn Gly Ala Asp Val Gln Val Ser Asn Ile Asp Tyr
785                 790                 795                 800

Arg Leu Ser Arg Lys Glu Leu Gln Gln Leu Leu Gln Glu Ala Phe Ala
                805                 810                 815

Arg His Gly Lys Val Lys Ser Val Glu Leu Ser Pro His Thr Asp Tyr
            820                 825                 830

Gln Leu Lys Ala Val Val Gln Met Glu Asn Leu Gln Asp Ala Ile Gly
        835                 840                 845

Ala Val Asn Ser Leu His Arg Tyr Lys Ile Gly Ser Lys Lys Ile Leu
    850                 855                 860

Val Ser Leu Ala Thr Gly Ala Ala Ser Lys Ser Leu Ser Leu Leu Ser
865                 870                 875                 880

Ala Glu Thr Met Ser Val Leu Gln Asp Ala Pro Ala Cys Cys Leu Pro
                885                 890                 895

Leu Phe Lys Phe Thr Asp Ile Tyr Glu Lys Lys Phe Gly His Lys Leu
            900                 905                 910

Asn Val Ser Asp Leu Tyr Lys Leu Thr Asp Thr Val Ala Ile Arg Glu
        915                 920                 925

Gln Gly Asn Gly Arg Leu Val Cys Leu Leu Pro Ser Ser Gln Ala Arg
    930                 935                 940

Gln Ser Pro Leu Gly Ser Ser Gln Ser His Asp Gly Ser Ser Thr Asn
945                 950                 955                 960

Cys Ser Pro Ile Ile Phe Glu Glu Leu Glu Tyr His Glu Pro Val Cys
                965                 970                 975

Arg Gln His Cys Ser Asn Lys Asp Phe Ser Glu His Glu Phe Asp Pro
            980                 985                 990

Asp Ser Tyr Lys Ile Pro Phe Val  Ile Leu Ser Leu Lys  Thr Phe Ala
        995                 1000                1005

Pro Gln  Val His Ser Leu Leu  Gln Thr His Glu Gly  Thr Val Pro
    1010                1015                1020

Leu Leu  Ser Phe Pro Asp Cys  Tyr Ile Ala Glu Phe  Gly Asp Leu
    1025                1030                1035

Glu Val  Val Gln Glu Asn Gln  Gly Gly Val Pro Leu  Glu His Phe
    1040                1045                1050
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Ile Thr  Cys Val Pro Gly Val  Asn Ile Ala Thr Ala  Gln Asn Gly
    1055                1060                1065

Ile Lys  Val Val Lys Trp Ile  His Asn Lys Pro Pro  Pro Pro Asn
    1070                1075                1080

Thr Asp  Pro Trp Leu Leu Arg  Ser Lys Ser Pro Val  Gly Asn Pro
    1085                1090                1095

Gln Leu  Ile Gln Phe Ser Arg  Glu Val Ile Asp Leu  Leu Lys Ser
    1100                1105                1110

Gln Pro  Ser Cys Val Ile Pro  Ile Ser His Phe Ile  Pro Ser Tyr
    1115                1120                1125

His His  His Phe Ala Lys Gln  Cys Arg Val Ser Asp  Tyr Gly Tyr
    1130                1135                1140

Ser Lys  Leu Ile Glu Leu Leu  Glu Ala Val Pro His  Val Leu Gln
    1145                1150                1155

Ile Leu  Gly Met Gly Ser Lys  Arg Leu Leu Thr Leu  Thr His Arg
    1160                1165                1170

Ala Gln  Val Lys Arg Phe Thr  Gln Asp Leu Leu Lys  Leu Leu Lys
    1175                1180                1185

Ser Gln  Ala Ser Lys Gln Val  Ile Val Arg Glu Phe  Ser Gln Ala
    1190                1195                1200

Tyr His  Trp Cys Phe Ser Lys  Asp Trp Asp Val Thr  Glu Tyr Gly
    1205                1210                1215

Val Cys  Glu Leu Ile Asp Ile  Val Ser Glu Ile Pro  Asp Thr Thr
    1220                1225                1230

Ile Cys  Leu Ser Gln Gln Asp  Asn Glu Met Val Ile  Cys Ile Pro
    1235                1240                1245

Lys Arg  Glu Arg Thr Gln Asp  Glu Ile Glu Arg Thr  Lys Gln Phe
    1250                1255                1260

Ser Lys  Asp Val Val Asp Leu  Leu Arg His Gln Pro  His Phe Arg
    1265                1270                1275

Met Pro  Phe Asn Lys Phe Ile  Pro Ser Tyr His His  His Phe Gly
    1280                1285                1290

Arg Gln  Cys Lys Leu Ala Tyr  Tyr Gly Phe Thr Lys  Leu Leu Glu
    1295                1300                1305

Leu Phe  Glu Ala Ile Pro Asp  Thr Leu Gln Val Leu  Glu Cys Gly
    1310                1315                1320

Glu Glu  Lys Ile Leu Thr Leu  Thr Glu Val Glu Arg  Phe Lys Ala
    1325                1330                1335

Leu Ala  Ala Gln Phe Val Lys  Leu Leu Arg Ser Gln  Lys Asp Asn
    1340                1345                1350

Cys Leu  Met Met Thr Asp Leu  Leu Thr Glu Tyr Ala  Lys Thr Phe
    1355                1360                1365

Gly Tyr  Thr Phe Arg Leu Gln  Asp Tyr Asp Val Ser  Ser Ile Ser
    1370                1375                1380

Ala Leu  Thr Gln Lys Leu Cys  His Val Val Lys Val  Ala Asp Ile
    1385                1390                1395

Glu Ser  Gly Arg Gln Ile Gln  Leu Ile Asn Arg Lys  Ser Leu Arg
    1400                1405                1410

Ser Leu  Thr Ala Gln Leu Leu  Val Leu Leu Met Ser  Trp Glu Gly
    1415                1420                1425

Thr Thr  His Leu Ser Val Glu  Glu Leu Lys Arg His  Tyr Glu Ser
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    1430                1435                1440

Thr His  Asn Thr Pro Leu Asn  Pro Cys Glu Tyr Gly  Phe Met Thr
    1445                1450                1455

Leu Thr  Glu Leu Leu Lys Ser  Leu Pro Tyr Leu Val  Glu Val Phe
    1460                1465                1470

Thr Asn  Asp Lys Met Glu Glu  Cys Val Lys Leu Thr  Ser Leu Tyr
    1475                1480                1485

Leu Phe  Ala Lys Asn Val Arg  Ser Leu Leu His Thr  Tyr His Tyr
    1490                1495                1500

Gln Gln  Ile Phe Leu His Glu  Phe Ser Met Ala Tyr  Thr Lys Tyr
    1505                1510                1515

Val Gly  Glu Thr Leu Gln Pro  Lys Thr Tyr Gly His  Ser Ser Val
    1520                1525                1530

Glu Glu  Leu Leu Gly Ala Ile  Pro Gln Val Val Trp  Ile Lys Gly
    1535                1540                1545

His Gly  His Lys Arg Ile Val  Val Leu Lys Asn Asp  Met Lys Ser
    1550                1555                1560

Arg Leu  Ser Ser Leu Ser Leu  Ser Pro Ala Asn His  Glu Asn Gln
    1565                1570                1575

Pro Ser  Glu Gly Glu Arg Ile  Leu Glu Val Pro Glu  Ser His Thr
    1580                1585                1590

Ala Ser  Glu Leu Lys Leu Gly  Ala Asp Gly Ser Gly  Pro Ser His
    1595                1600                1605

Thr Glu  Gln Glu Leu Leu Arg  Leu Thr Asp Asp Ser  Pro Val Asp
    1610                1615                1620

Leu Leu  Cys Ala Pro Val Pro  Ser Cys Leu Pro Ser  Pro Gln Leu
    1625                1630                1635

Arg Pro  Asp Pro Val Ile Leu  Gln Ser Ala Asp Leu  Ile Gln Phe
    1640                1645                1650

Glu Glu  Arg Pro Gln Glu Pro  Ser Glu Ile Met Ile  Leu Asn Gln
    1655                1660                1665

Glu Glu  Lys Met Glu Ile Pro  Ile Pro Gly Lys Ser  Lys Thr Leu
    1670                1675                1680

Thr Ser  Asp Ser Ser Ser Ser  Cys Ile Ser Ala Ala  Val Pro Val
    1685                1690                1695

Pro Pro  Cys Pro Ser Ser Glu  Thr Ser Glu Ser Leu  Leu Ser Lys
    1700                1705                1710

Asp Pro  Val Glu Ser Pro Ala  Lys Lys Gln Pro Lys  Asn Arg Val
    1715                1720                1725

Lys Leu  Ala Ala Asn Phe Ser  Leu Ala Pro Ile Thr  Lys Leu
    1730                1735                1740
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1. A method of treating a retroviral infection in a human in need of such treatment, comprising delivering a functional meiosis arrest female protein 1 (MARF1) to cells containing a retroviral provirus.

2. The method of claim **1**, wherein the MARF1 is delivered by means of a DNA vector comprising an expression cassette having a coding sequence encoding a functional MARF1 operatively linked to a promoter, that which will express MARF1 in said cells containing a retroviral provirus.

3. The method of claim **2** wherein the viral vector is a replication-deficient adenoviral (AV) vector or a replication-deficient adeno-associated viral (AAV) vector.

4. The method of claim **1** wherein the retroviral infection is human immunodeficiency virus (HIV).

5. The method of claim **1** wherein the functional meiosis arrest female protein 1 (MARF1) is delivered to the cells containing a retroviral provirus ex vivo, by removing from the patient's body, treating, and returning the cells containing a retroviral provirus.

6. A DNA vector comprising an expression cassette having a coding sequence encoding a functional MARF1 operatively linked to a heterologous promoter.

7. The vector of claim **6** which is a replication-deficient adenoviral (AV) vector or a replication-deficient adeno-associated viral (AAV) vector.

8. A packaging cell line which expresses a vector according to claim **6**.

9.-**10**. (canceled)

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