B16F10 tumor model mice.

Description of Embodiments

[0020] “Cytokine(s)” used herein is a polypeptide(s)/glycoprotein(s) derived from a natural lymphokine (cytokines made by lymphocytes), monokine (cytokines made by monocytes), chemokine (cytokines with chemotactic activities), an interleukin (cytokines made by one leukocyte and acting on other leukocytes), or modification thereof. The modified cytokine may be a fragment of the naturally occurring cytokine. In one embodiment, the modified cytokine has at least 70%, 75%, 80%, 85%, 90%, 95% or 98% amino acid sequence identity to a naturally occurring cytokine. In one embodiment, the modified cytokine is a mutant where at most 10% of the amino acids are deleted, substituted, and/or added based on a naturally occurring cytokine.

[0021] Examples of cytokines include, but are not limited to, interleukin (IL) including over 30 type such as IL-1α, IL-1β, IL-2, -3, -4, -5, -6, -7, -8, -9, -10, -11, -12, -13 to -37; interferon (IFN) such as IFN-α, IFN-β and IFN-γ; tumor necrosis factor (TNF) such as TNF-α and TNF-β; transforming growth factor (TGF) such as TGF-α and TGF-β; colony stimulating factor (CSF) such as granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage-colony stimulating factor (GM-CSF), macrophage-colony Stimulating factor (M-CSF), erythropoietin (EPO), stem cell factor (SCF) and monocyte chemotactic and activating factor (MCAF); growth factor (GF) such as epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin like growth factor (IGF), nerve growth factor (NGF), Brain-derived neurotrophic factor (BDNF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), thrombopoietin (TPO), and bone morphogenic protein (BMP); and other polypeptide factors including LIF, kit ligand (KL), MPO (Myeloperoxidase) and CRP (C-reactive protein) ; COX (Cyclooxygenase) such as COX-1, COX-2 and COX-3, NOS (Nitric oxide synthase) such as NOS-1, NOS-2 and NOS-3; and modified thereof.

[0022] Cytokines also includes chemokines which are cytokines that induce chemotaxis. There are two major classes of chemokines, CXC and CC. The CXC chemokines, such as neutrophil-activating protein-2 (NAP-2) and melanoma growth stimulatory activity protein (MGSA) are chemotactic primarily for neutrophils and T lymphocytes, whereas the CC chemokines, such as RANTES, Macrophage inflammatory protein (MIP) including MIP-lα and MIP-lβ, keratinocyte-derived chemokine (KC), the monocyte chemotactic proteins (MCP-1, MCP-2, MCP-3, MCP-4, and MCP-5) and the eotaxins (-1 and -2) are chemotactic for, among other cell types, macrophages, T lymphocytes, eosinophils, neutrophils, dendritic cells, and basophils. There also exist the chemokines lymphotactin-1, lymphotactin-2 (both C chemokines), and fractalkine (a CX3C chemokine) that do not fall into either of the major chemokine subfamilies.

[0023] Preferable example of a cytokine is, but not limited to, IL-12.

[0024] In the alphavirus replicon vector of the present disclosure, the gene encoding a cytokine may be fused to a gene encoding a transmembrane domain. As used herein, "transmembrane domain (TM)" is a protein derived either from a natural or from a synthetic source. Where the source is natural, the domain in some aspects is derived from any membrane-bound or transmembrane protein. In one aspect, the membrane-bound or transmembrane protein is a protein heterologous to the cytokine. Examples of the membrane-bound or transmembrane proteins may include the alpha, beta or zeta chain of a T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CDS, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154; toll-like receptors (TLR) such as TLR1-TLR10 in human and TLR1-TLR9, TLR11-TLR13 in mouse; interleukin (IL) receptors such as IL-1-28 receptor, RANTES receptors (CCR1, CCR3, CCR5), MIP-1 receptor, PF4 receptor, M-CSF receptor and NAP-2 receptor belonging to GPCR chemokine receptor; hemagglutinin (HA).

[0025] Examples of transmembrane proteins may also include the following:

5-Lipoxygenase-Activating Protein, ABC Transporters, ACBP, Amyloid beta (A4), Bcl-2 Inhibitors, BNIPs, CAAX protease, Cytochromes P450, E-NPPs, EPHA1, EPHA2, EPHA3, EPHA4, Fatty Acid Desaturases, Gamma secretase, Glucose transporter, Glycophorins, GPCR, HER2/ErbB2, HER3/ErbB3, HER4/ErbB4, HSD-11β, Hypoxia-induced Proteins, Immunoglobulins, Insulin receptor, Integrins, Ion channel, MAPEG, MFS, MinK Family, MPPs, Peptidase AD, Peptidase Family M48, Peptidase MA, Protein Jagged, Receptor-type Kinases, SNARE Complex, Sulfatases, TNF receptor, Transmembrane Proteins 14, Transporter, TROBP, VEGF receptors, Aldehyde Dehydrogenases, Ammonia and Urea transporters, FMN-linked Oxidoreductases, Leucine Rich Repeat (LRR)-Containing Transmembrane Proteins, Leukotriene C4 synthase, Lysosome-associated membrane glycoprotein, Major Intrinsic Protein (MIP)/FNT superfamily, Microsomal prostaglandin E synthase, N-(deoxy)ribosyltransferase-like Membrane Proteins, Neutral/alkaline Ceramidases, Oligosaccharyl Transferase, Pentameric Ligand-gated Ion Channels, Rhodopsin-like receptors and pumps, Single-helix ATPase Regulators, Squalene/ phytoene Synthase, Stearoyl -CoA desaturase 1, Stannin (SNN) Membrane Proteins, T-cell Surface Glycoprotein CD3 Zeta Chain, Tetratricopeptide repeat (TPR) Alpha-Helical Repeat Proteins, Transmembrane Proteins with NAD(P)-binding Rossmann-fold Domains.

[0026] In addition, monotypic/peripheral proteins that attached to the lipid bilayer or other integral proteins and peptide may also be used as transmembrane proteins. Examples may include Alpha/Beta-Hydrolase, Annexins, Bet V1-Like Protein, C1 Domain-Containing Protein, C2 Domain-containing Protein, CoA-Dependent Acyltransferases, CRAL-TRIO Domain-Containing Protein, DNase I-like protein, Fibrinogen , FYVE/PHD Zinc Finger Protein, Galactose-Binding Domain-Like Protein,

Glycolipid Transfer Protein, Immunoglobulin-Like Superfamily (E Set) Protein, Lipocalin, Lipoxygenase, PGBD superfamily, PH Domain-Like Protein, Phosphatidylinositol 3-/4-Kinase, PLC-like Phosphodiesterase, Phosphotyrosine Protein Phosphatases II, P-Loop Containing Nucleoside Triphosphate Hydrolase, Protein kinase superfamily , PX Domain-Containing Protein, Saposin , Synuclein and Transcriptional factor tubby.

[0027] The expression "transmembrane domain" used in the present disclosure includes at least transmembrane region(s) of the membrane-bound or transmembrane protein. In addition, the transmembrane domain may also include juxtamembrane domain (JMD) and/or cytoplasmic tail of the membrane-bound or transmembrane protein.

[0028] Alternatively the transmembrane domain in some embodiments is synthetic. In some aspects, the synthetic transmembrane domain comprises predominantly hydrophobic residues such as leucine and valine. There are also highly charged residues flanking the transmembrane domain (stop transport signals). In some aspects, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain.

[0029] Preferable examples of transmembrane domain may be those derived from TLR4 (Toll-like receptor 4) and influenza virus hemagglutinin (HA). Specific examples may include a protein consisting of the flexible juxtamembrane region or flexible linker, the transmembrane domain and the cytoplasmic tail of Influenza virus hemagglutinin "HA (flexible-TM-Cyt)" and a protein consisting of TLR4 (Toll-like receptor 4).

[0030] By fusing the gene encoding a cytokine with gene encoding a transmembrane domain, especially with the influenza virus hemagglutinin transmembrane domain, the safety of the resulting alphavirus replicon vector will be improved while keeping the effect to induce immunological activities.

[0031] A cytokine polypeptide and a transmembrane domain may be directly or indirectly fused. In one embodiment, one or two linkers may intervene between them.

[0032] Also a cytokine polypeptide and a transmembrane domain can be truncated and replaced by short linkers. In some embodiments, a cytokine structural protein and a heterologous transmembrane domain include one or more peptide linkers.

[0033] An example of a short linker consists of from 2 to 25 amino acids (e.g. 2, 3, 4, 5 or 6 amino acids). Usually, it is from 2 to 15 amino acids in length, such as SG, GS, SGG, GGS SGSG and TRGGS. In certain circumstances, the linker can be only one, such as glycine(G), serine (S) and cysteine (C).

[0034] When a cytokine polypeptide is chemically conjugated to a heterologous trans-

Claims

[Claim 1]

An alphavirus replicon vector comprising, a polynucleotide which encodes alphavirus non-structural proteins nsp1, nsp2, nsp3 and nsp4, and a polypeptide comprising a cytokine.

[Claim 2]

The alphavirus replicon vector of claim 1, wherein the cytokine is a lymphokine, monokine, chemokine, or interleukin.

[Claim 3]

The alphavirus replicon vector of claim 1, wherein the cytokine is an interleukin.

[Claim 4]

The alphavirus replicon vector of claim 3, wherein the interleukin is interleukin-12 (IL-12).

[Claim 5]

The alphavirus replicon vector of any one of claims 1-4, wherein the cytokine is fused to a transmembrane domain.

[Claim 6]

The alphavirus replicon vector of claim 5, wherein the transmembrane domain is derived from Influenza Hemagglutinin (HA) or TLR4.

[Claim 7]

The alphavirus replicon vector of claim 5 or 6, wherein the transmembrane domain is fused to the cytokine by a linker.

[Claim 8]

The alphavirus replicon vector of claim 7, wherein the linker is IgG4CH3.

[Claim 9]

The alphavirus replicon vector of any one of claims 1-8, which comprises a promoter, 5' UTR, polynucleotide encoding alphavirus non-structural proteins nsp1, nsp2, nsp3 and nsp4, SG promoter, a gene of interest encoding the polypeptide comprising a cytokine, 3'UTR and poly A tail.

[Claim 10]

The alphavirus replicon vector of any one of claims 1-9, wherein the polypeptide comprising a cytokine has the amino acid sequence of SEQ ID NO: 14 or 18.

[Claim 11]

The alphavirus replicon vector of any one of claims 1-10, wherein the alphavirus is a CHIKV or VEEV.

[Claim 12]

The alphavirus replicon vector of claims 11, wherein the CHIKV is CHIKV strain 37997 or strain OPY-1.

[Claim 13]

The alphavirus replicon vector of claims 12, wherein the VEEV is VEEV strain TC-83.

[Claim 14]

A composition comprising

(i) a pharmaceutically acceptable carrier, and

(ii) a vector of any one of claims 1-13.

[Claim 15]

The composition of claim 14, wherein the pharmaceutically acceptable carrier is a delivery vehicle and the alphavirus replicon vector is encapsulated in the delivery vehicle.

[Claim 16]

The composition of claim 15, wherein the delivery vehicle is a particle consisting of alphavirus structural proteins or a lipid delivery system.

[Claim 17]

The composition of claim 16, wherein the alphavirus structural proteins comprise a capsid and at least one envelope proteins and the capsid has one or more alterations in the Nuclear Localization Signal (NLS).

[Claim 18]

The composition of claim 16, wherein the alphavirus structural proteins comprise at least one envelope protein E3 which comprises one or more alterations at the furin site in the E3 protein.

[Claim 19]

A composition comprising a lipid nanoparticle and the alphavirus replicon vector of any one of claims 1-13, wherein the alphavirus replicon vector is encapsulated in the lipid nanoparticle.

[Claim 20]

A vaccine comprising the alphavirus replicon vector or composition according to any one of Claims 1-19.

[Claim 21]

A method of treating and/or immunizing against cancer or inflammatory disease in a subject, which comprises administering an effective amount of the alphavirus replicon vector or composition of any one of Claims 1-18 to the subject in need thereof.

[Claim 22]

Use of the alphavirus replicon vector or composition of any one of claims 1-18 for the manufacture of a medicament for treating and/or immunizing against cancer or inflammatory disease.

[Claim 23]

The alphavirus replicon vector or composition of any one of claims 1-18 for use in treating and/or immunizing against cancer or inflammatory disease.