[0004] Prior to Applicants' inventions, PRG4 had been appreciated as a protein with only mechanical properties, providing mechanical functionalities such as lubricating joints, tendons, cartilage, and acting as a mechanical barrier to inhibit intercellular interactions. However, as shown herein, Applicants have discovered that lubricin has properties that extend beyond its ability to provide boundary lubrication and anti-adhesion. In particular, Applicants have determined that PRG4 has anti-inflammatory properties due to its ability to act as a ligand or signaling molecule, participating in ligand receptor interactions to modulate, for example, CD44 activation, NF-κB translocation, and cytokine-mediated inflammation.

SUMMARY

[0004a] In an aspect of the present disclosure, there is provided a use of a proteoglycan 4 (PRG4) in the manufacture of a medicament for treating inflammatory bowel disease in a patient, wherein the proteoglycan 4 (PRG4) reduces or inhibits an inflammatory response associated with the inflammatory bowel disease.

[0005] The present disclosure exploits the heretofore unknown anti-inflammatory properties of PRG4, also known as lubricin. Accordingly, the present disclosure provides, for example, methods of inhibiting or reducing inflammatory responses and methods for treating inflammatory conditions. Underlying the discovery of PRG4's anti-inflammatory properties is the understanding of various putative mechanisms by which PRG4 achieves its anti-inflammatory effect. These mechanisms were discovered by Applicants who have determined that PRG4 binds CD44 receptors, enabling it to act as a CD44 receptor antagonist. As a result, PRG4 is able to down-regulate pro-inflammatory responses mediated by CD44 receptor signaling. The ability of PRG4 to affect CD44 signaling also has the effect of down-regulating translocation of NF-κB. Further, administration of PRG4 has been shown to inhibit production of a number of pro-inflammatory cytokines, as well as to modulate cellular responses and proliferation due to pro-inflammatory cytokine induction (e.g., TNF-a); a feature unique to PRG4 and not to other lubricants such as high molecular weight hyaluronic acid. PRG4 can therefore be used in a number of novel ways in therapeutic and prophylactic contexts to effect anti-inflammatory action via its effect on signaling pathways involved in producing an inflammatory response.

[0006] Accordingly, in one example, the present disclosure provides a method of reducing or inhibiting an inflammatory response in a patient comprising administering PRG4 to the patient systemically or locally at a site that is non-cartilaginous, non-osseous, non-osteal, and non-articular, and is not the urinary bladder, cornea, or surface tissue of the oral cavity.

[0007] In one example, the PRG4 is administered systemically to the patient, for example, by intravenous, intramuscular, subcutaneous, intraperitoneal, oral, rectal, buccal, or sublingual administration, or by inhalation.

[0008] In another example of the present disclosure, PRG4-containing compositions can be conveniently administered to patients by formulating PRG4 into a matrix/scaffold dosage form for injection/or placement within a location in a patient. Such a PRG4-containing composition may be in the form of a controlled release formulation that is capable of slowly releasing PRG4 at a location in the patient. Suitable matrix/scaffold dosage forms include, but are not limited to, biocompatible polymers, polymeric matrices, capsules, microcapsules, microparticles, diffusion devices and liposomes. Other such formulations of the present disclosure include liquids that, upon association with the matrix or upon administration to the patient, form a solid or a gel. In addition to such compositions being formulated to contain PRG4, such compositions may also be formulated to contain recombinantly engineered cells designed to express PRG4. The PRG4-containing compositions may be administered in any manner suitable to direct PRG4 to the location within a patient, including by direct injection or placement of a pre-formed PRG4 composition during an open surgery or during a laparoscopic or arthroscopic procedure.

[0009] In another example, the PRG4 is administered locally to the patient, for example, topically or by injection. In some examples, the PRG4 is administered locally at a site selected from skin, kidney, lungs, liver, a wound such as a skin burn or surgical incision, thyroid, pancreas, spleen, thymus, ovary, testicle, uterus, adrenal gland, pituitary gland, hypothalamus, urethra, prostate, heart, artery or vessel, pericardial fluid, brain, stomach. Administration is also conducted in orifices including the rectum, nose, ear, pharynx, larynx, trachea. Other areas for administration include the tongue, posterior eye, or the site of a tumor. Administration is also performed in visci including the small intestine, large intestine, colon or esophagus. , pharynx, larynx, trachea, tongue, posterior eye, or the site of a tumor. In some examples, the site of local administration is a site of inflammation or of an inflammatory response in the patient.

[0010] In another example, PRG4 is administered systemically to a patient who suffers from an inflammatory condition selected from arthritis, osteoarthritis, psoriatic arthritis,

rheumatoid arthritis, diabetic retinopathy, retinal inflammation, retinitis, Sjogren's syndrome, macular degeneration, gout, pseudogout, pericarditis, or uveitis.

[0011] In another example, PRG4 is administered systemically or locally to a patient who suffers from an inflammatory condition selected from acne; acute organ failure; acute respiratory distress syndrome (ARDS); Addison's disease; allergic rhinitis; allograft rejection; alopecia areata; Alzheimer's disease; anaphylaxis; Appendicitis; asthma; atherosclerosis; atopic dermatitis; autoimmune disease including autoimmune alopecia; autoimmune hyperthyroidism; autoimmune hypopituatarism; autoimmune polyglandular disease; Behcet's disease; brain injury; bronchitis; cancer; cardiopulmonary bypass syndrome; cardiorenal syndrome; Celiac disease; chronic actinic dermatitis; chronic obstructive pulmonary disease (COPD); chronic renal failure; colitis; contact dermatitis; Crohn's disease; dermatomyositis; diabetes; eczema; emphysema; foreign body rejection; glaucoma; glomerulonephritis; gout; graft vs. host disease; Graves' Disease; Guillain-Barre syndrome; Hashimoto's thyroiditis; hay fever; hepatorenal syndrome; hypersensitivity or allergy; inclusion body myositis; infection due to viral, fungal, parasitic or microbial infiltration; inflammatory bowel disease; inflammatory kidney disease; injury due to thermal or chemical exposure or irradiation; irritable bowel syndrome; ischemia; lung inflammation; morphea; multiple sclerosis; mycosis fungoides; myocardial infarction; necrosis; non-infectious lung injury; pancreatitis; pernicious anemia; pneumonia; polymyositis; prostatitis; pseudogout; psoriasis; Pustulosis palmoplantaris; Pyoderma gangrenum; respiratory allergy; scleroderma; sepsis; serum sickness; Sezary's syndrome; skin allergy; stroke; systemic inflammatory response syndrome (SIRS); systemic lupus erythematosus; systemic sclerosis; T-cell mediated hypersensitivity diseases; transplant rejection; trauma including from a bullet wound, knife wound, automobile accident, fall, or combat; tuberculosis; ulcerative colitis; pericarditis; uticaria; and vitiligo.

[0012] In a further example, the inflammatory response in the patient is associated with an inflammatory condition from which the patient suffers.

[0013] In a further example, the PRG4 administered is recombinant human PRG4. In another example, the PRG4 has the sequence of SEQ ID NO:1 minus the signal sequence. In one example, PRG4 is administered in an amount insufficient to provide

boundary lubrication in the patient. In another example, PRG4 is administered in an amount ranging from 0.1 μg/kg – 4,000 μg/kg or as a coating on a tissue surface applied from a PRG4 solution comprising, for example, a concentration of PRG4 between 10 μg/mL to about 2 mg/mL.

[0014] In one example, the reduction or inhibition of the inflammatory response may be measured by the level of production of a pro-inflammatory cytokine in the patient. For example, in one example, the pro-inflammatory cytokine is selected from the group consisting of IL-1α, IL-1β**,** IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-12p70, IL-13, IL-14, IL-15, IL-16, IL-17, IL-17α, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-34, IL-35, IL-36, TNF-α, TNF-β (lymphotoxin-α), lymphotoxin-β, CXC31L (Fractalkine), CXCL-8, CCL2, CCL3, CCL4, CCL5, CCL11, CXCL10, IFN-α, IFN-β, IFN-ε, IFN-κ, IFN-ω, IFN-γ, VEGF, MCP-1, MCP-3, EGF, GMCSF, CD40L, CD27L, CD30L, FASL, 4-1BBL, OX40L, TRAIL, FGF-2, GRO, MDC, Rantes, G-CSF, M-CSF, FGF-2, EPO, MCSF, MIP3α, MG-CSF, and GCSF.

[0015] In another example, the present disclosure provides a method of reducing or inhibiting an inflammatory response in a patient having an inflammatory condition, the method comprising administering PRG4 to the patient. The inflammatory condition may be any of the conditions mentioned herein.

[0016] In one example, the present disclosure provides a method of reducing or inhibiting an inflammatory response in a patient by administering PRG4 to the patient. Administration of PRG4 binds a CD44 receptor on a cell in the patient, reduces or inhibits the production of a pro-inflammatory cytokine in the patient, and/or reduces or inhibits the translocation of NF-κB in a cell in the patient and thereby reduces or inhibits the inflammatory response in the patient.

[0017] In another example, the PRG4 is administered locally to said patient to cells at or about a non-cartilaginous tissue, a non-osteal tissue, a non-osseous tissue, and is not corneal, urinary bladder or oral cavity tissue, which is a site of inflammation in the patient.

[0018] In a further example, the cell is a mast cell, a spleen cell, a lung cell, a renal cell, a brain cell, a cardiac cell, a hepatic cell, a cancer cell, a skin cell, an epithelial cell, an endothelial cell, leukocyte, lymphocyte, neutrophil, eosinophil, basophil, monocyte,

macrophage, dendritic cell, fibroblast, muscle cell, urethral cell, vascular cell, nerve cell, pancreatic cell, gastric cell, intestinal cell, colon cell, rectal cell, gall bladder cell, stem cell, or thyroid cell.

[0019] In a further example, PRG4 is administered to the patient systemically to contact a synoviocyte, a chondrocyte, an osteocyte, an osteoblast, an osteoclast, retinal cell, limbal cell, trabecular meshwork cell, corneal cell, conjunctival cell, an ocular cell, or an ophthalmic cell.

[0020] In yet another example, whether PRG4 is administered locally or systemically, it is administered in an amount insufficient to provide boundary lubrication in the patient. For example, in one example, the PRG4 is administered in amount ranging from 0.1 μg/kg – 4,000 μg/kg.

[0021] In a further example, administration of PRG4 reduces or inhibits a pro-inflammatory cytokine selected from the group consisting of IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-γ, TNF-α, IL-1α, IL-1β, MCP-1, EGF, FGF-2, Fractalkine, IFN-α2, GRO, MCP-3, MDC, EPO, IL-13, IL-18, MCSF, MIP-3α, MG-CSF, IL-7, IL-5, G-CSF, Rantes, IL-17α, or IL-12p70.

[0022] In another example, the present disclosure provides a method of inhibiting binding of an activating ligand to CD44 present on a surface. The method comprises exposing the surface to PRG4 at a concentration sufficient to bind CD44 and to inhibit binding of the ligand. According to one example, PRG4 is present at the surface in an amount sufficient to bind CD44 but insufficient to provide boundary lubrication. In another example, the surface is a mammalian cell membrane while in another example the surface is in a surface plasmon resonance detector. In yet another example, the PRG4 is rhPRG4 (recombinant human PRG4) or nhPRG4 (native human PRG4).

[0023] In a further example, the surface is in a human subject. In another example, the PRG4 is administered systemically. In another example, the PRG4 is administered topically to a patient. In yet another example, the PRG4, for example rhPRG4, is administered to a human subject in amount ranging from 0.1 μg/kg – 4,000 μg/kg. In another example, the PRG4, for example, rhPRG4, is administered in an amount of 0.1

μg/mL to 30 mg/mL in small volumes of 1-100 μL per dose. In another example, the PRG4 is administered in an amount of 10 μg/mL to 4 mg/mL in volumes of 100 μL – 4 L per dose, for instance as an enema.

[0024] In one example, when the surface is in a human subject, the human suffers from a bone metabolic disorder and exposing the receptor to PRG4 reduces or inhibits osteoclast differentiation. In a further example, the human suffers from an inflammatory condition. Exemplary inflammatory conditions have been listed herein above. In one example, exposing the receptor to PRG4, for example, rhPRG4 reduces inflammation or the level of a pro-inflammatory cytokine in the location of the inflammatory condition.

[0025] According to one example, the cell is a synoviocyte, a mast cell, a spleen cell, a lung cell, a renal cell, a brain cell, a cardiac cell, an eye cell, a hepatic cell, a cancer cell, a skin cell, an epithelial cell, or an endothelial cell. In a further example, the cell is a synoviocyte of a subject with rheumatoid arthritis, a pancreatic cell of a diabetic, a lung cell of an asthmatic, an ocular cell of a subject with an eye infection, burn or other irritation; a bronchiolar or alveolar epithelial cell of a subject with tuberculosis or another lung infection or injury or condition; a lung cell of a cystic fibrosis subject; a lower intestinal epithelial cell of a person with colitis or Crohn's disease; a skin cell of a subject with psoriasis, a skin cell of a subject with acne; a skin cell of a subject following treatment with laser ablation, or an endothelial cell of subject with sepsis. In yet another example, the cell is a T-cell, while in a further example, the cell is a lymphocyte, neutrophil, fibroblast, cancer cell, macrophage, dendritic cell, monocyte, eosinophil, or endothelial cell.

[0026] According to one example, exposing a surface to PRG4, for example, rhPRG4, antagonizes a pro-inflammatory ligand of CD44. In one example, the ligand is hyaluronan (HA), a hyaluronan serum-derived hyaluronan-associated protein complex (HA-SHAP), or a matrix metalloproteinase (e.g., MMP-9). In another example, the ligand is hemopexin, EMMPRIN, somatomedin-B, osteopontin, OKT3, or a complement related protein such as C3a, CD3, CD46.

[0027] In another example, the present disclosure provides a method of reducing or inhibiting pro-inflammatory cytokine levels in blood, for example, in a human subject. The method comprises the step of administering PRG4 systemically in an amount sufficient to reduce or

inhibit pro-inflammatory cytokine levels. Exemplary proinflammatory cytokines include IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-γ, TNF-α, IL1-α, IL-1-β, MCP-1, EGF, FGF-2, fractalkine, IFN-α2, GRO, MCP-3, MDC, EPO, IL-13, IL-18, MCSF, MIP-3α, MG-CSF, IL-7, IL-5, G-CSF, Rantes, IL-17α, or IL-12p70.

[0028] In a further example, the present disclosure provides a method of inhibiting NF-κB translocation in a cell. The method comprises the step of contacting a cell containing NF-κB with PRG4, wherein PRG4 binds to a cell surface receptor to inhibit activation of the NF-κB signaling pathway. In a further example, PRG4 inhibits a TNF-α receptor or an IL-1 receptor on the cell surface.

[0029] In yet another example, the cell is in a human when contacted with PRG4. In a further example, the cell is a synoviocyte, chondrocyte, or osteocyte, while in yet another example, the cell is a spleen cell, a lung cell, a renal cell, a brain cell, a cardiac cell, a brain cell, a hepatic cell, an epithelial cell or an endothelial cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] **FIGS. 1A-C** are bar graphs presenting data demonstrating the binding of recombinant human proteoglycan 4 (rhPRG4), high-molecular weight hyaluronic acid (HMW HA), and medium molecular weight hyaluronic acid (MMW HA) to recombinant human CD44 receptor as detected by TMB-ELISA at 450 nm. Data represents the average of 4 independent experiments with triplicate wells per group. **FIG. 1A** depicts binding of rhPRG4, HMW HA, MMW HA and vitronectin to CD44-IgG1Fc and using IgG1 Fc. The star (*) indicates that the 450 nm absorbance in the CD44-IgG1 Fc wells were statistically significantly higher ($p<0.001$) than the IgG1 Fc wells for rhPRG4, HMW HA and MMW HA. **FIG. 1B** shows the concentration-dependent CD44 binding of rhPRG4, HMW HA and MMW HA. CD44 binding to rhPRG4 was significantly higher than to HMW HA or MMW HA ($p<0.001$). The double stars (**) indicate that CD44 binding to rhPRG4 was significantly higher than to MMW HA ($p<0.001$). **FIG. 1C** depicts the competition between rhPRG4 (5 μg/mL) and either HMW HA or MMW HA (0.01 μg/mL to 50 μg/mL) on binding to CD44 coated on 96-well ELISA plates. The star (*) indicates the percentage CD44 binding in HMW HA+rhPRG4 wells was significantly lower than rhPRG4 wells ($p<0.05$); (**) indicates the percentage CD44 binding in MMW HA+rhPRG4 wells was significantly lower than rhPRG4 wells ($p<0.05$).

What is claimed is:

1. Use of proteoglycan 4 (PRG4) in the manufacture of a medicament for treating inflammatory bowel disease in a patient, wherein the proteoglycan 4 (PRG4) reduces or inhibits an inflammatory response associated with the inflammatory bowel disease.

2. The use of claim 1, wherein the PRG4 is to be administered systemically to the patient.

3. The use of claim 2, wherein the PRG4 is to be administered intravenously, intraperitoneally, by inhalation, intramuscularly, subcutaneously, orally, rectally, buccally, or sublingually.

4. The use of claim 1, wherein the PRG4 is to be administered locally to the gastrointestinal tract of the patient.

5. The use of claim 4 wherein the PRG4 is to be administered locally to the rectum, the colon, the small intestine, the stomach, or a combination thereof.

6. The use of any one of claims 4 or 5, wherein the PRG4 is to be administered by injection.

7. The use of any one of claims 4 or 5, wherein the PRG4 is to be administered orally.

8. The use of claim 7, wherein the PRG4 is to be administered orally into the gastrointestinal tract of the patient as a tablet, a capsule, as a solution by ingestion, or by feeding tube.

9. The use of claim 8, wherein the tablet or capsule comprises a substance that degrades when exposed to conditions present in the gastrointestinal tract.

10. The use of any one of claims 1-6, wherein the PRG4 is to be administered rectally.

11. The use of claim 10, wherein the PRG4 is to be administered by enema.

12. The use of any one of claims 1-5, wherein the PRG4 is to be administered via a colostomy.

13. The use of any one of claims 1-5, wherein the PRG4 is to be administered to the small intestine or large intestine by J-tube.

14. The use of claim 11, wherein the PRG4 is to be administered in an amount of 100 mL to 4L in a concentration ranging from 10 μg/mL to 200 μg/mL.

15. The use of any one of claims 1-14, wherein a laxative is to be first administered followed by fasting before the PRG4 is to be administered.

16. The use of any one of claims 1-15, wherein the PRG4 is exogenous human PRG4.

17. The use of any one of claims 1-15, wherein the PRG4 is recombinant human PRG4.

18. The use of any one of claims 1-17, wherein the PRG4 comprises the amino acid sequence of residues 25-1404 of SEQ ID NO:1.

19. The use of any one of claims 1-18, wherein the PRG4 is to be administered in an amount insufficient to provide boundary lubrication in the patient.

20. The use of any one of claims 1-19, wherein the PRG4 is to be administered in an amount ranging from 0.1 μg/kg - 4000 μg/kg.

21. The use of any one of claims 1-20, wherein the inflammatory bowel disease is Crohn’s disease or ulcerative colitis.