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(54) **USE OF BMP ANTAGONISTS TO REGULATE HEPCIDIN-MEDIATED IRON METABOLISM**

(57) The present invention provides new systems and strategies for the regulation of iron metabolism in mammals. In particular, methods of using agonists and antagonists of TGF- β superfamily members to modulate the expression or activity of hepcidin, a key regulator of iron metabolism, are described. The inventive methods find applications in the treatment of diseases associated with iron overload, such as juvenile hemochromatosis

and adult hemochromatosis, and in the treatment of diseases associated with iron deficiency, such as anemia of chronic disease and EPO resistant anemia in end-stage of renal disease. The present invention also relates to screening tools and methods for the development of novel drugs and therapies for treating iron metabolism disorders.

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Description**Related Applications**

[0001] The present application claims priority to Provisional Application No. 60/653,479 filed on February 16, 2005 and entitled "Methods and Compositions to Regulate Iron Metabolism". The Provisional Application is incorporated herein by reference in its entirety.

Background of the Invention

[0002] Iron is an essential element for the growth and survival of nearly all living organisms (P. Aisen et al., J. Biochem. Cell Biol., 2001, 33: 940-959) except for a few unusual bacterial species. It plays an important role in oxygen transport and storage (in combination with oxygen-binding molecules such as hemoglobin and myoglobin) and is a key component of many enzymes that catalyze the redox reactions required for the generation of energy (e.g., cytochromes), the production of various metabolic intermediates, and for host defense (e.g., nicotinamide adenine dinucleotide phosphate [NADPH] oxidase). Iron can also be toxic. It catalyzes the generation of reactive radical species that can attack cellular membranes, proteins, and DNA (J.M.C. Gutteridge et al., Biochem. J., 1982, 296: 605-609), and activates NF- κ B, the prototype transcription factor for genes involved in inflammation (S. Xiong et al., J. Biol. Chem., 2003, 278: 17646-17654). At high levels, iron accumulation in tissues is damaging.

[0003] To prevent iron deficiency or iron overload, virtually all organisms have developed elaborate mechanisms for regulating iron intake and efflux (C. Finch, Blood, 1994, 84: 1697-1702). In adult mammals, iron homeostasis depends upon regulated absorption of iron by the enterocyte, a highly specialized cell of the duodenum that coordinates dietary iron uptake and transport into the body. In the fetus, the mechanisms involved in placental materno-fetal iron transport are also tightly regulated. Iron is stored in the body in the form of the protein complexes, ferritin and hemosiderin, and is transported in the plasma *via* the protein complex, transferrin. Under normal circumstances, only trace amounts of iron exist outside these physiologic sinks, although stored iron can be mobilized by reuse. Perturbations in these highly regulated mechanisms can lead to iron overload or iron deficiency in the body.

[0004] Iron deficiency is the most common nutritional disorder in the world. As many as 4-5 billion people (*i.e.*, 65-80% of the world's population) may be iron deficient; and 2 billion people (over 30% of the world's population, mostly children and women of childbearing age) are anemic, mainly due to iron deficiency. In developing countries, the disease is exacerbated by malaria and worm infections. Iron deficiency affects more people than any other condition, constituting a public health condition of epidemic proportions. Iron overload disorders are less prevalent; however, they can lead to serious life-threatening conditions. Worldwide, some 24 million people of northern European ancestry suffer from a genetic disorder called hemochromatosis. Another 600 million carry one of the genes responsible for the disorder, and absorb up to 50% more iron than non-carriers. The disease leads to iron accumulation, particularly in the liver and other storage organs, which can cause organ failure (like cirrhosis of the liver), heart attack, cancer, and pancreatic damage.

[0005] Dysfunctions in iron metabolism pose a major problem worldwide due not only to their frequency but also to the lack of therapeutic options (N.C. Andrews, N. Engl. J. Med., 1999, 341: 1986-1995). Iron overload conditions are generally treated by administration of iron chelating agents, which exert their effects by remobilizing accumulated iron and allowing for its excretion. In practice, however, none of the chelating agents which have been evaluated to date have proved entirely satisfactory, suffering from poor gastrointestinal absorption, and either low efficacy, poor selectivity, or undesirable side effects. The preferred treatment for reducing iron levels in most hemochromatosis patients is called therapeutic phlebotomy, a procedure which simply consists of removing blood from the body. Patients with hemochromatosis usually need a large number of phlebotomies in a relative short period of time (up to once or twice a week). Thus, in addition to carrying the same risks as with any blood donation (*e.g.*, nausea, vomiting, dizziness, fainting, hematoma, seizures or local infection), phlebotomy can also be highly constraining to the patient.

[0006] Several forms of iron salt are used to treat iron deficiency conditions. It generally takes several months of replacement therapy to replenish body iron stores. Some patients have difficulty tolerating iron salts, because these substances tend to cause gastrointestinal distress. Studies have also reported that liquid iron-salt preparations, given to young children, may cause permanent staining of the teeth. However, more problematic is the finding that high doses of iron supplements, taken orally or by injection, can increase susceptibility to bacterial infection.

[0007] Clearly, the development of novel agents and methods for the prevention and treatment of iron metabolism disorders, remains highly desirable.

Summary of the Invention

[0008] The present invention provides improved systems and strategies for regulating iron metabolism in mammals, including humans. In particular, the invention encompasses reagents and processes for the treatment of conditions

associated with iron deficiency or iron overload. The invention also provides screening tools and methods for the identification of compounds useful for the treatment of iron metabolism disorders. Compared to existing therapies such as iron supplementation, iron chelation, and phlebotomy, the inventive methods and compositions are less likely to induce undesirable side-effects.

[0009] In general, the present invention involves the use of modulators of the signaling activity of members of the TGF- β superfamily to control and/or regulate the expression or activity of hepcidin, a key regulator of iron metabolism in mammals.

[0010] More specifically, in one aspect, the present invention provides methods for regulating hepcidin expression or activity in a subject by administering to the subject an effective amount of a compound that modulates the signaling activity of at least one TGF- β superfamily member. The present invention also provides methods for regulating hepcidin expression or activity in a biological system by contacting the biological system with an effective amount of a compound that modulates the signaling activity of at least one TGF- β superfamily member. In certain embodiments, the TGF- β superfamily member is TGF- β or BMP. The compound administered to the subject or contacted with the biological system may comprise an agent selected from the group consisting of an agonist of TGF- β , an antagonist of TGF- β , an agonist of BMP, an antagonist of BMP, or combinations thereof. The biological system may be a cell, a biological fluid, a biological tissue or an animal.

[0011] In certain embodiments, the agent is selected from the group consisting of a HJV.Fc fusion protein, a Dragon.Fc fusion protein, a DLN.Fc fusion protein, a sT β R.II.Fc fusion protein, a sT β R.II-B.Fc fusion protein, and a sT β R.III Δ .Fc fusion protein. In some embodiments, the agent comprises a fusion protein selected from the group consisting of a mutant HJV.Fc fusion protein, a mutant Dragon.Fc fusion protein and mutant DLN.Fc fusion protein, wherein the mutant fusion protein is non-proteolytically cleavable.

[0012] In methods for inhibiting hepcidin expression or activity in a subject or a biological system, the compound administered to the subject or contacted with the biological system is preferably an agonist of TGF- β , an antagonist of BMP, or combinations thereof. In methods for enhancing hepcidin expression or activity in a subject or a biological system, the compound administered to the subject or contacted with the biological system is preferably an antagonist of TGF- β , an agonist of BMP, or combinations thereof.

[0013] In another aspect, the present invention provides methods for regulating iron metabolism or an iron metabolic process in a subject or a biological system by administering to the subject or contacting the biological system with an effective amount of a compound that modulates the signaling activity of at least one TGF- β superfamily member. The iron metabolic process may be iron uptake, iron absorption, iron transport, iron storage, iron processing, iron mobilization, iron utilization, or combinations thereof.

[0014] When the subject or biological system exhibits or is at risk of exhibiting iron deficiency, the compound used in these methods is preferably an agonist of TGF- β , an antagonist of BMP, or combinations thereof. When the subject or biological system exhibits or is at risk of exhibiting iron overload, the compound is, preferably, an antagonist of TGF- β , an agonist of BMP, or combinations thereof.

[0015] In still another aspect, the present invention provides methods for treating or preventing conditions associated with perturbations in iron metabolism in a subject. The inventive methods comprise administering to the subject an effective amount of a compound that modulates the signaling activity of at least one TGF- β superfamily member. In certain embodiments, the TGF- β superfamily member is TGF- β or BMP. In some embodiments, administration of the compound to the subject results in regulation of hepcidin expression or activity in the subject.

[0016] Compounds administered in the inventive methods may comprise an agent selected from the group consisting of an agonist of TGF- β , an antagonist of TGF- β , an agonist of BMP, an antagonist of BMP, or combinations thereof. In certain embodiments, the agent is selected from the group consisting of a HJV.Fc fusion protein, a Dragon.Fc fusion protein, a DLN.Fc fusion protein, a sT β R.II.Fc fusion protein, a sT β R.II-B.Fc fusion protein, and a sT β R.III Δ .Fc fusion protein. In some embodiments, the agent comprises a fusion protein selected from the group consisting of a mutant HJV.Fc fusion protein, a mutant Dragon.Fc fusion protein and mutant DLN.Fc fusion protein, wherein the mutant fusion protein is non-proteolytically cleavable.

[0017] When the subject has or is at risk of having a condition associated with iron deficiency, the compound used in these methods is, preferably, an antagonist of BMP, an agonist of TGF- β , or combinations thereof. Conditions associated with iron deficiency that can be treated and/or prevented by methods of the present invention include, but are not limited to, anemia of chronic disease, iron deficiency anemia, functional iron deficiency, and microcytic anemia. In certain embodiments, the methods further comprise administering an iron supplementation treatment to the subject.

[0018] When the subject has or is at risk of having a condition associated with iron overload, the compound used in the methods of treatment is, preferably, an agonist of BMP, an antagonist of TGF- β , or combinations thereof. Conditions associated with iron overload that can be treated and/or prevented by methods of the present invention include, but are not limited to, adult hemochromatosis and juvenile hemochromatosis. In certain embodiments, the methods further comprise administering an iron chelation treatment to the subject. In some embodiments, the methods further comprise performing phlebotomy to the subject.

[0019] In yet another aspect, the present invention provides methods for identifying compounds that regulate hepcidin expression or activity in a biological system, and methods for identifying compounds that regulate iron metabolism or an iron metabolic process in a biological system. In these methods, the biological system preferably expresses at least one TGF- β superfamily member.

[0020] These methods comprise incubating the biological system with a candidate compound under conditions and for a time sufficient for the candidate compound to modulate the signaling activity of the TGF- β superfamily member, thereby obtaining a test system; measuring, in the test system, at least one factor that is representative of the signaling activity of the TGF- β superfamily member; incubating the system under the same conditions and for the same time absent the candidate compound, thereby obtaining a control system; measuring the factor in the control system; comparing the factor measured in the test and control systems; and determining that the candidate compound regulates hepcidin expression or iron metabolism in the system, if the factor measured in the test system is less than or greater than the factor measured in the control system.

[0021] In certain embodiments, the TGF- β superfamily member is TGF- β or BMP, and the compound identified as regulator is selected from the group consisting of an agonist of TGF- β , an antagonist of TGF- β , an agonist of BMP, and an antagonist of BMP. In some embodiments, the agent comprises a fusion protein selected from the group consisting of a mutant HJV.Fc fusion protein, a mutant Dragon.Fc fusion protein and mutant DLN.Fc fusion protein, wherein the mutant fusion protein is non-proteolytically cleavable.

[0022] The present invention also provides pharmaceutical compositions comprising a pharmaceutically acceptable carrier, and an effective amount of at least one regulator of iron metabolism or at least one regulator of hepcidin expression or activity identified by the inventive screening methods. Also provided are methods of using these identified regulators in the treatment or prevention of conditions associated with perturbations in iron metabolism.

Brief Description of the Drawing

[0023]

Figure 1 is a graph showing the effects of BMP-2, Noggin.Fc (which is known to inhibit endogenous BMP signaling), and Fe-NTA (non-transferrin-bound iron) on the hepcidin/actin ratio in HepG2 cells compared to the hepcidin/actin ratio in control cells, C (*i.e.*, HepG2 cells incubated in the absence of these agents).

Figure 2 is a graph showing the effects of BMP-2, Noggin.Fc, and TGF- β 1 on the hepcidin/actin ratio in HepG2 cells compared to the hepcidin/actin ratio in control cells, C (*i.e.*, HepG2 cells incubated in the absence of these agents).

Figure 3 shows a set of Western blot analyses of HJV protein in the liver (A) and transfected CHO cells (B), and of soluble HJV.Fc fusion protein (C).

Figure 4 is a set of three graphs showing measurements of luciferase activity in HepG2 cells transfected with a BMP-responsive luciferase reporter (A, C) or a TGF- β responsive luciferase reporter (B) incubated with or without BMP-2, BMP-4 or TGF- β 1.

Figure 5 is a graph showing measurements of luciferase activity in HepG2 cells transfected with BMP-responsive luciferase reporter and HJV cDNA or empty vector, and incubated with or without exogenous BMP-2 in the presence or absence of Noggin.

Figure 6(A) is a graph showing results of radioactivity measurements from HJV.Fc incubated with ^{125} I-labeled BMP-2. **Figure 6(B)** is a gel showing that ^{125} I-BMP-2 can be chemically crosslinked with HJV.Fc in the presence of DSS (bar 4) and that this crosslinking can be inhibited by excess cold BMP-2 (bar 5).

Figure 7 is a graph reporting radioactivity measurements from HJV.Fc incubated with ^{125} I-labeled BMP-2 with or without excess cold BMP-2, -4, -7, or TGF- β 1.

Figure 8 is a set of two graphs showing measurements of luciferase activity in HepG2 cells co-transfected with BMP-responsive luciferase reporter and HJV either alone or in combination with dominant negative BMP type I receptor ALK3 (ALK3 DN) or ALK6 (ALK6 DN) (**Fig. 8(A)**), or with wild-type (WT) versus dominant negative (DN) R-Smad 1 (**Fig. 8(B)**).

Figure 9 shows two Western blot analyses of mutant HJVG313V (**Fig. 9(A)**) and soluble HJVG313V.Fc cDNA (**Fig.**

9(B)) made using PCR and subcloning techniques, and transfected into CHO cells

Figure 10 shows immunofluorescence microscopy images of unpermeabilized cells transfected with wild-type HJV and mutant HJVG313V.

Figure 11 is a graph reporting measurements of luciferase activity in HepG2 cells transfected with BMP-responsive luciferase reporter alone or in combination with increasing concentrations of wild-type HJV or mutant HJVG13V cDNA.

Figure 12 is a table reporting measurements of serum iron and total iron binding capacity in mice after intraorbital treatment with BMP-2 ligand.

Figure 13 is a gel showing that mouse RMGa-D169A.Fc fusion protein is not proteolytically cleaved compared to mouse RMGa.Fc fusion protein.

Figure 14 is a gel showing that mouse Dragon-D171A.Fc fusion protein is not proteolytically cleaved compared to mouse Dragon.Fc fusion protein.

Figure 15 is a gel showing that human HJV-D172A is not proteolytically cleaved compared to human HJV.

Definitions

[0024] Throughout the specification, several terms are employed that are defined in the following paragraphs.

[0025] As used herein, the term "*a TGF- β superfamily member*" refers to any member of the TGF- β superfamily, which includes, among others, activins, inhibins, Transforming Growth Factors beta (TGF- β s), Growth and Differentiation Factors (GDFs), Bone Morphogenetic Proteins (BMPs), and Müllerian Inhibiting Substance (MIS). In the context of the present invention, certain preferred TGF- β superfamily members include TGF- β s and BMPs.

[0026] The term "*perturbations*" when applied to iron metabolism or an iron metabolic process, refers to any disturbances, dysregulations and/or deviations from normal state, function and/or level of activity. Iron metabolic processes include iron uptake, iron absorption, iron transport, iron storage, iron processing, iron mobilization, and iron utilization. Generally, perturbations in iron metabolism result in iron overload or iron deficiency. As used herein, the term "*iron overload*" refers to an amount of iron present in a subject's tissue or in a biological system which is significantly above the normal level in that particular tissue or that particular biological system. The term "*iron deficiency*" refers to an amount of iron present in a subject's tissue or in a biological system which is significantly below the normal level in that particular tissue or that particular biological system. An amount of iron significantly below or significantly above the normal level corresponds to any amount of iron that is physiologically undesirable and/or that is or may become harmful to the subject or the biological system. Methods for the determination of iron levels are known in the art (see below).

[0027] As used herein, the term "*condition associated with perturbations of iron metabolism or an iron metabolic process*" refers to any disease, disorder, syndrome or condition that is characterized by iron overload or iron deficiency.

[0028] The term "*prevention*" is used herein to characterize a method that is aimed at delaying or preventing the onset of a pathophysiological condition associated with perturbations in iron metabolism (for example in a subject which may be predisposed to the condition but has not yet been diagnosed as having it).

[0029] The term "*treatment*" is used herein to characterize a method that is aimed at (1) delaying or preventing the onset of a condition associated with perturbations in iron metabolism; or (2) slowing down or stopping the progression, aggravation, or deterioration of the symptoms of the condition; or (3) bringing about ameliorations of the symptoms of the condition; or (4) curing the condition. A treatment may be administered prior to the onset of the disease, for a prophylactic or preventive action. It may also be administered after initiation of the disease, for a therapeutic action.

[0030] The terms "*compound*" and "*agent*" are used herein interchangeably. They refer to any naturally occurring or non-naturally occurring (*i.e.*, synthetic or recombinant) molecule, such as a biological macromolecule (*e.g.*, nucleic acid, polypeptide or protein), organic or inorganic molecule, or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian, including human) cells or tissues. The compound may be a single molecule, a mixture of two or more molecules, or a complex of at least two molecules.

[0031] The term "*candidate compound*" refers to a compound or agent (as defined above) that is to be tested for an activity of interest. In certain screening methods of the invention, candidate compounds are evaluated for their ability to regulate hepcidin expression and/or to regulate iron metabolism through modulation of the signaling activity of a TGF- β superfamily member.

[0032] The term "*regulation*" when applied to a biological phenomenon (such as iron metabolism, and hepcidin expression or activity) refers to a process that allows for control of the biological phenomenon. The term "regulation" also

refers to the ability of a compound to control the biological phenomenon. For example, a process or a compound that regulates iron metabolism has the ability to decrease iron levels in a subject or biological system that exhibits iron overload; and/or the ability to increase iron levels in a subject or biological system that exhibits iron deficiency. In the context of the present invention, the mechanism by which regulation of the biological phenomenon takes place is preferably through modulation of the signaling activity of a TGF- β superfamily member. In the screening methods of the invention, when a candidate compound is found to regulate hepcidin expression or activity, it is identified as a "*regulator*" of the expression or activity of hepcidin.

[0033] As used herein, the term "*modulation of the signaling activity of a TGF- β superfamily member*" refers to the ability of a compound to increase or prolong, or to decrease or reduce the duration of the effect of a TGF- β superfamily member. In the screening methods of the invention, when a candidate compound is found to induce such an enhancement or inhibition, it is identified as a "*modulator*" of the signaling activity of the TGF- β superfamily member.

[0034] The term "*agonist*" is intended to be used as is accepted in the art. In general, the term refers to a compound that increases or prolongs the duration of the effect of a polypeptide or a nucleic acid. An agonist may be a direct agonist, in which case it is a molecule that exerts its effect by interacting with (e.g., binding to) the polypeptide or nucleic acid, or an indirect agonist, in which case it exerts its effect *via* a mechanism other than by interaction with the polypeptide or nucleic acid (e.g., by altering the expression or stability of the polypeptide or nucleic acid, by altering the expression or activity of a target of the polypeptide or nucleic acid, by interacting with an intermediate in a pathway involving the polypeptide or nucleic acid, etc.).

[0035] The term "*antagonist*" is intended to be used as is accepted in the art. In general, the term refers to a compound that decreases or reduces the duration of the effect of a polypeptide or a nucleic acid. An antagonist may be a direct antagonist, in which case it is a molecule that exerts its effect by interacting with (e.g., binding to) the polypeptide or nucleic acid, or an indirect antagonist, in which case it exerts its effect *via* a mechanism other than by interaction with the polypeptide or nucleic acid (e.g., by altering the expression or stability of the polypeptide or nucleic acid, by altering the expression or activity of a target of the polypeptide or nucleic acid, by interacting with an intermediate in a pathway involving the polypeptide or nucleic acid, etc.).

[0036] As used herein, the term "*effective amount*" refers to any amount of a compound or agent that is sufficient to fulfill its intended purpose(s). In the context of the present invention, the purpose(s) may be, for example: to modulate the signaling activity of a TGF- β superfamily member; and/or to regulate hepcidin expression or activity; and/or to regulate iron metabolism or an iron metabolic process; and/or to delay or prevent the onset of a condition associated with perturbations in iron metabolism; and/or to slow down or stop the progression, aggravation, or deterioration of the symptoms of the condition; and/or to bring about ameliorations of the symptoms of the condition; and/or to cure the condition.

[0037] The term "*subject*" refers to a human or another mammal, that can be affected by a pathophysiological condition associated with perturbations in iron metabolism but may or may not have such a condition.

[0038] The terms "*system*" and "*biological system*" are used herein interchangeably. A system may be any biological entity that can exhibit iron overload or iron deficiency. The biological system is preferably one that expresses at least one TGF- β superfamily member. Some preferred systems express TGF- β and/or BMP. The biological system may also preferably express hepcidin or comprise hepcidin. In the context of this invention, *in vitro*, *in vivo*, and *ex vivo* systems are considered; and the system may be a cell, a biological fluid, a biological tissue, or an animal. A system may, for example, originate from a live subject (e.g., it may be obtained by drawing blood, or by biopsy), or from a deceased subject (e.g., it may be obtained at autopsy).

[0039] As used herein, the term "*biological fluid*" refers to a fluid produced by and obtained from a subject. Examples of biological fluids include, but are not limited to, urine, blood serum, and plasma. In the present invention, biological fluids include whole or any fraction of such fluids derived by purification, for example, by ultra-filtration or chromatography.

As used herein, the term "*biological tissue*" refers to a tissue obtained from a subject. The biological tissue may be whole or part of any organ or system in the body (e.g., liver, gastrointestinal tract, kidney, pancreas, and the like).

[0040] A "*pharmaceutical composition*" is defined herein as comprising at least one compound of the invention (*i.e.*, a candidate compound identified by an inventive screening method as a regulator of iron metabolism, and/or a regulator of hepcidin expression or activity), and at least one pharmaceutically acceptable carrier.

[0041] As used herein, the term "*pharmaceutically acceptable carrier*" refers to a carrier medium which does not interfere with the effectiveness of the biological activity of the active ingredients and which is not excessively toxic to the hosts at the concentrations at which it is administered. The term includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic agents, absorption delaying agents, and the like. The use of such media and agents for the formulation of pharmaceutically active substances is well known in the art (see, for example, Remington's Pharmaceutical Sciences, E.W. Martin, 18th Ed., 1990, Mack Publishing Co., Easton, PA).

Detailed Description of Certain Preferred Embodiments

[0042] As mentioned above, the present invention provides improved systems and strategies for regulating iron metabolism in mammals. In particular, the inventive compounds and methods are less likely than existing therapies to induce undesirable side effects.

I. Agonists and Antagonists of TGF- β Superfamily Members as Regulators of Iron Metabolism

[0043] The present invention encompasses the discovery that certain members of the TGF- β superfamily can regulate the expression of hepcidin, a key regulator of iron metabolism in mammals. As described in Example 1, the present Applicants have recognized that BMP (bone morphogenetic protein) signaling induces hepcidin expression in HepG2 liver hepatoma cells, while TGF- β (Transforming Growth Factor-beta) signaling inhibits the expression of hepcidin. Furthermore, using Noggin, a well-known BMP antagonist, the Applicants have shown that inhibition of BMP signaling resulted in reduction of hepcidin expression.

[0044] Accordingly, the present invention provides methods of using agonists and/or antagonists of members of the TGF- β superfamily to regulate hepcidin expression or activity, which, in turn, regulates iron metabolism.

A - Hepcidin

[0045] Hepcidin is a small, cysteine-rich cationic peptide with antimicrobial properties that was purified only recently from human urine and plasma ultra-filtrate (C.H. Park et al., J. Biol. Chem., 2001, 276: 7806-7810; A. Krause et al., FEBS Lett., 2000, 480: 147-150). This peptide of 20, 22 or 25 amino acids, differing by amino acid terminal truncation, forms a short hairpin with two arms linked by four disulfide bridges in a ladder-like fashion. Hepcidin contains eight cysteine residues that are conserved among species (G. Nicolas et al., Proc. Natl. Acad. Sci. USA, 2001, 98: 878-885; C. Pigeon et al., J. Biol. Chem., 2001, 276: 7811-7819). Even though the peptide was first isolated from urine and blood, hepcidin is predominantly expressed in the liver in both mice and humans. Expression is also detectable in the heart and brain, but to a much less extent (C.H. Park et al., 2001; C. Pigeon et al., 2001). Recently, hepcidin has also been found to be expressed in the kidney (H. Kulaksiz et al., J. Endocrinol., 2005, 184: 361-370).

[0046] Only one copy of the gene exists in humans, whereas two hepcidin genes (Hepc1 and Hepc 2) have been reported in mice (G. Nicolas et al., Proc. Natl. Acad. Sci. USA, 2001, 98: 878-885; C. Pigeon et al., J. Biol. Chem., 2001, 276: 7811-7819). Both the human and mouse hepcidin genes consist of three exons and two introns, with the third exon encoding the mature peptide found in urine. In humans and rats, the exons encode an 84 (83 in mice) amino acid precursor, including a putative 24 amino acid signal peptide.

[0047] The connection between hepcidin and iron metabolism was first made by Pigeon et al. (J. Biol. Chem., 2001, 276: 7811-7819) while investigating hepatic responses to iron overload. Other studies have shown that mice lacking hepcidin mRNA developed iron overload affecting liver and pancreas, with deficit in the macrophage-rich spleen (G. Nicolas et al., Proc. Natl. Acad. Sci. USA, 2001, 98: 8780-8785). Transgenic mice overexpressing hepcidin were observed to die at birth of severe iron deficiency (G. Nicolas et al., Proc. Natl. Acad. Sci. USA, 2002, 99: 4596-4601). These studies suggested that hepcidin inhibits iron absorption in the small intestine, the release of recycled iron from macrophages (R.E. Fleming and W.S. Sly, Proc. Natl. Acad. Sci. USA, 2001, 98: 8160-8162), and transport of iron across the placenta (G. Nicolas et al., Proc. Natl. Acad. Sci. USA, 2002, 99: 4596-4601). In agreement with animal studies, patients with large hepatic adenomas and otherwise unexplained iron refractory anemia exhibit overexpressed hepcidin mRNA in the liver (D.A. Weinstein et al., Blood, 2002, 100: 3776-3781). Recent studies have found abnormal hepcidin expression and disrupted hepcidin regulation (K.R. Bridle et al., Lancet, 2003, 361: 669-673; H. Kulaksiz et al., Gut, 2004, 53: 735-743) in hemochromatosis gene (HFE)-associated hemochromatosis and association of hepcidin mutations with severe juvenile hemochromatosis (A. Roetto et al., Nature Genetics, 2003, 33: 21-22). Based on these and other observations, it has been suggested that hepcidin is a key component of iron homeostasis that acts as a negative regulator of iron metabolism.

B - TGF- β Superfamily Members

[0048] The TGF- β superfamily of ligands presently comprises more than 30 members, including, among others, activins, inhibins, Transforming Growth Factors-beta (TGF- β s), Growth and Differentiation Factors (GDFs), Bone Morphogenetic Proteins (BMPs), and Müllerian inhibiting Substance (MIS). All of these molecules are peptide growth factors that are structurally related to TGF- β . They all share a common motif called a cysteine knot, which is constituted by seven especially conservative cysteine residues organized in a rigid structure (J. Massagué, Annu. Rev. Biochem., 1998, 67: 753-791). Unlike classical hormones, members of the TGF- β superfamily are multifunctional proteins whose effects depend on the type and stage of the target cells as much as the growth factors themselves.

[0049] TGF- β superfamily members suitable for use in the practice of the methods of the present invention include any member of the TGF- β superfamily whose signaling activity can regulate the expression or activity of hepcidin. Preferred TGF- β superfamily members include, but are not limited to, TGF- β s and BMPs.

Transforming Growth Factors-beta (TGF- β s)

[0050] In certain embodiments of the invention, the TGF- β superfamily member is TGF- β . TGF- β s are extracellular polypeptides that are implicated in a broad range of biological processes (J. Massagué, *Ann. Rev. Cell. Biol.*, 1990, 6: 597-641) and play a central role in key events during embryogenesis, adult tissue repair, and immunosuppression (M.B. Sporn and A.B. Roberts, *J. Cell. Biol.* 1992, 119: 1017-1021; S.W. Wahl, *J. Clin. Immunol.* 1992, 12: 61-74; D.M. Kingsley, *Genes Dev.* 1994, 8: 133-146). In mammals, TGF- β is produced by almost all cells of the organism, and almost all cells can serve as targets for its effects. TGF- β is a potent regulator of cell proliferation, cell differentiation, apoptosis, and extracellular matrix production.

[0051] Mammalian cells can produce three different isoforms of TGF- β : TGF- β 1, TGF- β 2, and TGF- β 3. These isoforms exhibit the same basic structure (they are homodimers of 112 amino acids that are stabilized by intra- and inter-chain disulfide bonds) and their amino acid sequences present a high degree of homology (> 70%). However, each isoform is encoded by a distinct gene, and each is expressed in both a tissue-specific and developmentally regulated fashion (J. Massagué, *Annu. Rev. Biochem.* 1998, 67: 753-791). According to modern concepts, TGF- β exerts its effects by first binding to membrane receptors on the target cell, thereby initiating downstream signaling events. Cross-linking studies have shown that TGF- β mainly binds to three high-affinity cell-surface proteins, called TGF- β receptors of type I, type II, and type III (J. Massagué and B. Like, *J. Biol. Chem.* 1985, 260: 2636-2645; S. Cheifetz et al., *J. Biol. Chem.* 1986, 261: 9972-9978).

[0052] Regulation of iron metabolism according to methods of the present invention may be achieved by inhibition or enhancement of the signaling activity of any one of the isoforms of TGF- β (*i.e.*, TGF- β 1, TGF- β 2, and TGF- β 3) as long as this inhibition or enhancement results in regulation of hepcidin expression or activity.

Bone Morphogenetic Proteins (BMPs)

[0053] In other embodiments of the present invention, the TGF- β superfamily member is BMP. BMPs were originally identified as proteins that induce bone formation at ectopic (*i.e.*, non-skeletal) sites (A.H. Reddi, *Curr. Opin. Genet. Dev.*, 1994, 4: 737-744). However, it is now clear that in addition to their roles in bone and cartilage morphogenesis, BMPs are also involved in prenatal development and postnatal growth and/or repair of the eye, heart, blood, lung, kidney, muscle, skin, and other tissues (K.A. Waite and C. Eng, *Nat. Rev. Genet.*, 2003, 4: 763-773). Studies have shown that BMPs play an important role in regulating proliferation, apoptosis, differentiation, and chemotaxis of various cell types, including mesenchymal cells, epithelial cells, hematopoietic cells and neuronal cells. (J. Massagué and Y.G. Chen, *Genes Dev.*, 2000, 14: 627-644; K. Miyazono et al., *J. Cell Physiol.*, 2001, 187: 265-276; N. Morrell et al., *Circulation*, 2001, 104: 790-795; A. von Budnoff and K.W.Y. Cho, *Dev. Biol.*, 2001, 239: 1-14).

[0054] In a manner similar to other members of the TGF- β superfamily, BMPs mediate their effects by forming a complex of two different types of transmembrane serine/threonine kinase receptors: type I and type II (C.H. Heldin et al., *Nature*, 1997, 390: 465-471; J. Newman et al., *N. Engl. J. Med.*, 2001, 345: 319-324; B.L. Rosenzweig et al., *Proc. Natl. Acad. Sci. USA*, 1995, 92: 7632-7636). Three different BMP type I receptors (activin receptor-like kinase ALK2, ALK3, and ALK6) and three BMP type II receptors (BMP type II receptor (BMPRII); Activin type IIA receptor (ActRIIA); and Activin type IIB receptor (ActRIIB)) have been identified (L. Attisano and J.L. Wrana, *Science*, 2002, 296: 1646-1647). BMP binding induces phosphorylation of the type I receptor by the type II receptor, which leads to phosphorylation of cytoplasmic receptor-activated Smads (C.H. Heldin et al., *Nature*, 1997, 390: 465-471).

[0055] To date, nearly 20 BMP isoforms have been identified and characterized in mammals and newer ones are being discovered (M. Kawabata et al., *Cytokine Growth Factor Rev.*, 1998, 9: 49-61). The BMP family members have been classified in subgroups according to how closely they are related to each other structurally (T. Sakou, *Bone*, 1998, 22: 591-603; R.G. Schaub and J. Wozney, *Curr. Opin. Biotechnol.*, 1991, 2: 868-871; J.M. Schmitt et al., *J. Orthop. Res.*, 1999, 17: 269-278). *In vivo*, the BMP isoforms have different profiles of expression, different affinities for receptors and therefore unique biological activities.

[0056] Regulation of iron metabolism according to methods of the present invention may be achieved by inhibition or enhancement of the signaling activity of any one of the isoforms of BMP as long as this inhibition or enhancement results in regulation of hepcidin expression or activity.

C - Agonists and Antagonists of TGF- β Superfamily Members

[0057] Agonists and antagonists of a TGF- β superfamily member suitable for use in the methods of the present

invention include any compound or agent that has the ability to modulate (*i.e.*, enhance or inhibit) the signaling activity of the TGF- β superfamily member such that this modulation results in regulation of hepcidin expression or activity.

[0058] Suitable agonists and antagonists include naturally-occurring agonists and antagonists of the TGF- β superfamily member (including fragments and variants thereof that retain the biological characteristics of the naturally-occurring agonist and antagonist ligands). Suitable agonists and antagonists also include synthetic or human recombinant compounds. Classes of molecules that can function as agonists include, but are not limited to, small molecules, antibodies (including fragments or variants thereof, such as Fab fragments, Fab'2 fragments and scFvs), and peptidomimetics. Classes of molecules that can function as antagonists include, but are not limited to, small molecules, antibodies (including fragments or variants thereof), fusion proteins, antisense polynucleotides, ribozymes, small interfering RNAs (siRNAi), and peptidomimetics.

[0059] As will be appreciated by those skilled in the art, any compound or agent that is identified, for example, by the inventive screening assays (described below), as a modulator of a TGF- β superfamily member is suitable for use in the practice of methods of the present invention. In particular, small molecules modulators that exhibit high specificity may be of value in these methods.

Agonists and Antagonists of BMPs

[0060] Various antagonists of BMPs are known in the art (see, for example, G.J. Thomsen et al., Trends Genet., 1997, 13: 209-211; E. Canalis et al., Endocr. Rev. 2003, 24: 218-235; V.A. Botchkarev, J. Invest. Dermatol., 2003, 120: 36-47; U.S. Pat. No. 6,432,410, each of which is incorporated herein by reference in its entirety). In particular, the effects of BMPs can be modulated by a group of secreted polypeptides that prevent BMP signaling by binding BMPs, thereby precluding their binding to specific cell surface receptors. BMP antagonists suitable for use in the practice of the present invention include, but are not limited to, Noggin, chordin, ventroptin, follistatin and follistatin-related gene (FLRG). Other suitable BMP antagonists include cerberus, gremlin, caronte, DAN, Dante, and sclerostin and other structurally related proteins, which are collectively termed the DAN family (D. Hsu et al., Mol. Cell, 1998, 1: 673-683, which is incorporated herein by reference in its entirety). Proteins of the DAN family have a conserved cysteine-knot motif, which is also found in other growth factors, including TGF- β -like factors (J.J. Pearce et al., Dev. Biol., 1999, 209: 98-110; C.R. Rodriguez Esteban et al., Nature, 1999, 401: 243-251). However, other BMP antagonists lack sequence similarity with each other. *In vivo*, these BMP antagonists have distinct expression profiles, different affinities for various BMP isoforms, and regulate different biological responses.

[0061] The present invention also provides other BMP antagonists. As reported in Example 2, hemojuvelin (HJV) is a member of the repulsive guidance molecule (RGM) family of proteins. Individuals with HJV mutations are known to exhibit depressed levels of hepcidin. The present Applicants have shown that HJV enhances BMP but not TGF- β signaling. The results they obtained demonstrate that HJV binds directly to BMP-2; and that the enhancing effect of HJV on BMP signaling is reduced by administration of Noggin, indicating that HJV's action is ligand-dependent. Accordingly, a family of soluble HJV.Fc fusion proteins is provided herein as BMP antagonists suitable for use in the practice of the present invention.

[0062] The present Applicants have recently reported (T.A. Samad et al., "DRAGON: a bone morphogenetic protein co-receptor", J. Biol. Chem., 2005, 280: 14122-14129, which is incorporated herein by reference in its entirety) that DRAGON, a 436 amino acid glycosylphosphatidylinositol (GPI)-anchored member of the RGM family, which is expressed early in the developing nervous system, enhances BMP but not TGF- β signaling and acts as a BMP co-receptor. Accordingly, the present invention provides a family of soluble DRAGON.Pc fusion proteins as BMP antagonists suitable for use in the inventive methods. An example of a DRAGON.Fc fusion protein that can be used in the practice of the present invention has been described by the present Applicants (T.A. Samad et al., "DRAGON: A member of the repulsive guidance molecule-related family of neuronal- and muscle-expressed membrane proteins is regulated by DRG11 and has neuronal adhesive properties", J. Neuroscience, 2004, 24: 2027-2036, which is incorporated herein by reference in its entirety). Soluble DRAGON.Fc fusion protein has been found to bind selectively to BMP-2 and BMP-4, but not to BMP-7 or other members of the TGF- β superfamily of ligands (T.A. Samad et al., J. Biol. Chem., 2005, 280: 14122-14129).

[0063] Also provided herein is a family of RGMA.Fc (or DLN.Fc) fusion proteins as BMP antagonists suitable for use in the practice of the present invention. Like DRAGON, RGMA is a member of the repulsive guidance molecule (RGM) family of genes. RGMA and DRAGON are expressed in a complementary manner in the central nervous systems, where RGMA mediates repulsive axonal guidance and neural tube closure, while DRAGON contributes to neuronal cell adhesion through homophilic interactions. The present Applicants have shown that RGMA enhances BMP, but not TGF- β , signals in a ligand-dependent manner in cell culture and that the soluble extracellular domain of RGMA fused to human Fc (RGMA.Fc or DLN.Fc) forms a complex with BMP type 1 receptors and binds directly and selectively to radiolabeled BMP-2 and BMP-4 (J.L. Babitt et al., "Repulsive guidance molecule (RGMA), a DRAGON homologue, is a bone morphogenetic protein co-receptor", J. Biol. Chem., 2005, 280: 29820-29827, which is incorporated herein by reference in its entirety).

[0064] The present invention also provides mutant HJV, RGMa and DRAGON fusion proteins. In particular, mutant HJV, RGMa and DRAGON fusion proteins are provided that are more stable to proteolytic cleavage than the corresponding wild-type versions. It is known in the art that HJV, RGMa and DRAGON share a consensus proteolytic cleavage site. For human HJV, the cleavage site is situated after aspartic acid residue 172 (G. Papanikolaou et al., *Nature Genetics*, 2004, 36: 77-82, which is incorporated herein by reference in its entirety); for human DRAGON, after aspartic acid residue 168 (T.A. Samad et al., *J. Neuroscience*, 24: 2027-2036, which is incorporated herein by reference in its entirety); and for human RGMa, after aspartic acid residue 168 (Genbank Sequence # NM 020211).

[0065] Mutant HJV, RGMa and DRAGON fusion proteins of the present invention contain one mutation or more than mutation that confers stability to the fusion protein, in particular stability to proteolytic cleavage. For example, the aspartic acid residue situated close to the cleavage site may be substituted by a different residue or deleted. Alternatively or additionally, a residue in the vicinity of the cleavage site may be substituted by a different residue or deleted. Methods that allow specific mutations or mutations in specific portions of a polynucleotide sequence that encodes an isolated polypeptide to provide variants are known in the art. The present Applicants have demonstrated the feasibility of producing mutant HJV, RGMa, and DRAGON proteins that are not proteolytically cleaved, as reported in Example 4.

Agonists and Antagonists of TGF- β s

[0066] Multiple naturally-occurring modulators have been identified that enhance or inhibit TGF- β signaling. Access of TGF- β ligands to receptors is inhibited by the soluble proteins LAP, decorin and α 2-macroglobulin that bind and sequester the ligands (W. Balemans and W. Van Hul, *Dev. Biol.*, 2002, 250: 231-250). TGF- β ligand access to receptors is also controlled by membrane-bound receptors. BAMBI acts as a decoy receptor, competing with the type I receptor (D. Onichtchouk et al., *Nature*, 1999, 401: 480-485); betaglycan (TGF- β type II receptor) enhances TGF- β binding to the type II receptor (C.B. Brown et al., *Science*, 1999, 283: 2080-2082; J. Massagué, *Annu. Rev. Biochem.*, 1998, 67: 753-791; E. del Re et al., *J. Biol. Chem.*, 2004, 279: 22765-22772); and endoglin enhances TGF- β binding to ALK1 in endothelial cells (D.A. Marchuk, *Curr. Opin. Hematol.*, 1998, 5: 332-338; J. Massagué, *Nat. Rev. Mol. Cell Biol.*, 200, 1: 169-178; Y. Shi and J. Massagué, *Cell*, 2003, 113: 685-700). Cripto, an EGF-CFC GPI-anchored membrane protein, acts as a co-receptor, increasing the binding of the TGF- β ligands, nodal, Vg1, and GDF1 to activin receptors (S.K. Cheng et al., *Genes Dev.*, 2003, 17: 31-36; M.M. Shen and A.F. Schier, *Trends Genet.*, 2000, 16: 303-309) while blocking activin signaling.

[0067] Thus, agonists and antagonists of TGF- β signaling suitable for use in the practice of the methods of the present invention include naturally-occurring TGF- β antagonists (e.g., decorin, see, for example, Y. Yamaguchi et al., *Nature*, 1990, 346: 281-284, which is incorporated herein by reference in its entirety); soluble forms of naturally-occurring TGF- β agonists (e.g., a soluble form of endoglin, see, for example, U.S. Pat. Nos. 5,719,120; 5,830,847; and 6,015,693, each of which is incorporated herein by reference in its entirety); as well as inhibitors of naturally-occurring TGF- β antagonists.

[0068] Other suitable TGF- β antagonists include antagonists that have been developed to suppress undesired effects of TGF- β s for therapeutic purposes. For example, anti-TGF- β antibodies, whose dissociation constants have been reported to be in the nanomolar range have been described (U.S. Pat. No. 5,571,714, which is incorporated herein by reference in its entirety). These anti-TGF- β antibodies have been successfully administered to animals with diverse pathological conditions (W.A. Broder et al., *Nature*, 1990, 346: 371-374; S.W. Wahl, *J. Clin. Immunol.* 1992, 12: 61-74; M. Shah et al., *Lancet*, 1992, 339: 213-214; M.S. Steiner and E.R. Barrack, *Mol. Endocrinol.* 1992, 6: 15-25; F.N. Ziyadeh et al., *Proc. Natl. Acad. Sci. USA*, 2000, 97: 8015-8020).

[0069] Other TGF- β inhibitors have been developed based on an *in vitro* study, which showed that adenovirus-mediated transfer of a truncated TGF- β type II receptor completely and specifically abolishes diverse TGF- β signaling (H. Yamamoto et al., *J. Biol. Chem.* 1996, 271: 16253-16259, which is incorporated herein by reference in its entirety). Several of these truncated receptors possess potent antagonistic activity against their ligands by acting as dominant-negative mutants (A. Bandyopadhyay et al., *Cancer Res.* 1999, 59: 5041-5046; Z. Qi et al., *Proc. Natl. Acad. Sci. USA*, 1999, 96: 2345-2349; T. Nakamura et al., *Hepatol.* 2000, 32: 247-255, each of which is incorporated herein by reference in its entirety).

[0070] Soluble forms of TGF- β type II receptor (Sakamoto et al., *Gene Ther.* 2000, 7: 1915-1924; H. Ueno et al., *Gene Ther.* 2000, 11: 33-42; J. George et al., *Proc. Natl. Acad. Sci. USA*, 1999, 96: 12719-12724, each of which is incorporated herein by reference in its entirety) and type III receptor (PCT application No. PCT/US2004/014175 to the present Applicants, which is incorporated herein by reference in its entirety) have also been produced as fusion proteins and have successfully been used to prevent or treat TGF- β -related pathophysiological conditions in animal models.

II. Identification of Regulators of Iron Metabolism

[0071] In another aspect, the present invention provides methods for the identification of compounds that regulate iron metabolism by modulating the signaling activity of a TGF- β superfamily member. The present invention also provides methods for the identification of compounds that regulate hepcidin expression or activity by modulating the signaling

activity of a TGF- β superfamily member.

[0072] Preferably, these methods comprise incubating a biological system, which expresses at least one TGF- β superfamily member, with a candidate compound under conditions and for a time sufficient for the candidate compound to modulate the signaling activity of the TGF- β superfamily member, thereby obtaining a test system; incubating the biological system under the same conditions and for the same time absent the candidate compound, thereby obtaining a control system; measuring, in the test system, at least one factor that is representative of the signaling activity of the TGF- β superfamily member; measuring the factor in the control system; comparing the factor measured in the test and control systems; and determining that the candidate compound regulates hepcidin expression (and/or regulates iron metabolism), if the factor measured in the test system is less than or greater than the factor measured in the control system.

[0073] The screening methods provided herein will lead to the discovery and development of regulators of iron metabolism and regulators of hepcidin expression or activity that exert their effects by modulating the signaling activity of one or more TGF- β superfamily members. These regulators may be potentially useful in the treatment of conditions associated with perturbations in iron metabolism.

A - Biological Systems

[0074] The assay and screening methods of the present invention may be carried out using any type of biological systems, *i.e.*, a cell, a biological fluid, a biological tissue, or an animal. In certain embodiments, the system is a biological entity that can exhibit iron deficiency or iron overload (*e.g.*, an animal model, a blood sample, or whole or part of an organ, *e.g.*, the liver); and/or a biological entity that expresses at least one TGF- β family member (*e.g.*, a cell); and/or a biological entity that expresses hepcidin (*e.g.*, a hepatocyte) or comprises hepcidin (*e.g.*, a blood or urine sample).

[0075] In certain embodiments, the assay and screening methods of the present invention are carried out using cells that can be grown in standard tissue culture plastic ware. Such cells include all normal and transformed cells derived from any recognized sources. Preferably, cells are of mammalian (human or animal, such as rodent or simian) origin. More preferably, cells are of human origin. Mammalian cells may be of any organ or tissue origin (*e.g.*, brain, liver, blood, or kidney) and of any cell types. Suitable cell types include, but are not limited to, epithelial cells, platelets, lymphocytes, monocytes, myocytes, macrophages, hepatocytes, cardiomyocytes, endothelial cells, tumor cells, and the like.

[0076] Cells to be used in the practice of the assays and screening methods of the present invention may be primary cells, secondary cells, or immortalized cells (*e.g.*, established cell lines). They may be prepared by techniques well known in the art (for example, cells may be obtained by drawing blood from a patient or a healthy donor) or purchased from immunological and microbiological commercial resources (for example, from the American Type Culture Collection, Manassas, VA). Alternatively or additionally, cells may be genetically engineered to contain, for example, a gene of interest such as a gene expressing a growth factor or a receptor.

[0077] Selection of a particular cell type and/or cell line to perform an assay according to the present invention will be governed by several factors such as the nature of the TGF- β superfamily member whose signaling activity is to be modulated and the intended purpose of the assay. For example, an assay developed for primary drug screening (*i.e.*, first round(s) of screening) may preferably be performed using established cell lines, which are commercially available and usually relatively easy to grow, while an assay to be used later in the drug development process may preferably be performed using primary or secondary cells, which are often more difficult to obtain, maintain, and/or to grow than immortalized cells but which represent better experimental models for *in vivo* situations.

[0078] Examples of established cell lines that can be used in the practice of the assays and screening methods of the present invention include HepG2 liver hepatoma cells, Hep3B liver hepatoma cells, primary hepatocytes, and immortalized hepatocytes. Primary and secondary cells that can be used in the inventive screening methods, include, but are not limited to, epithelial cells, platelets, lymphocytes, monocytes, myocytes, macrophages, hepatocytes, cardiomyocytes, endothelial cells, and tumor cells.

[0079] Cells to be used in the inventive assays may be cultured according to standard cell culture techniques. For example, cells are often grown in a suitable vessel in a sterile environment at 37°C in an incubator containing a humidified 95% air-5% CO₂ atmosphere. Vessels may contain stirred or stationary cultures. Various cell culture media may be used including media containing undefined biological fluids such as fetal calf serum. Cell culture techniques are well known in the art and established protocols are available for the culture of diverse cell types (see, for example, R.I. Freshney, "Culture of Animal Cells: A Manual of Basic Technique", 2nd Edition, 1987, Alan R. Liss, Inc.).

[0080] In certain embodiments, the screening methods are performed using cells contained in a plurality of wells of a multi-well assay plate. Such assay plates are commercially available, for example, from Stratagene Corp. (La Jolla, CA) and Corning Inc. (Acton, MA) and include, for example, 48-well, 96-well, 384-well and 1536-well plates.

B - Candidate Compounds

[0081] As will be appreciated by those of ordinary skill in the art, any kind of compounds or agents can be tested using

the inventive methods. A candidate compound may be a synthetic or natural compound; it may be a single molecule, or a mixture or complex of different molecules. In certain embodiments, the inventive methods are used for testing one or more compounds. In other embodiments, the inventive methods are used for screening collections or libraries of compounds. As used herein, the term "*collections*" refers to any set of compounds, molecules or agents, while the term

"*library*" refers to any set of compounds, molecules or agents that are structural analogs.

[0082] Traditional approaches to the identification and characterization of new and useful drug candidates generally include the generation of large collections and/or libraries of compounds followed by testing against known or unknown targets (see, for example, WO 94/24314; WO 95/12608; M.A. Gallop et al., J. Med. Chem. 1994, 37: 1233-1251; and E.M. Gordon et al., J. Med. Chem. 1994, 37: 1385-1401). Both natural products and chemical compounds may be tested by the methods of the invention. Natural product collections are generally derived from microorganisms, animals, plants, or marine organisms; they include polyketides, non-ribosomal peptides, and/or variants thereof (for a review, see, for example, D.E. Cane et al., Science, 1998, 82: 63-68). Chemical libraries often consist of structural analogs of known compounds or compounds that are identified as hits or leads *via* natural product screening. Chemical libraries are relatively easy to prepare by traditional automated synthesis, PCR, cloning or proprietary synthetic methods (see, for example, S.H. DeWitt et al., Proc. Natl. Acad. Sci. U.S.A. 1993, 90:6909-6913; R.N. Zuckermann et al., J. Med. Chem. 1994, 37: 2678-2685; Carell et al., Angew. Chem. Int. Ed. Engl. 1994, 33: 2059-2060; P.L. Myers, Curr. Opin. Biotechnol. 1997, 8: 701-707).

[0083] Collections of natural compounds in the form of bacterial, fungal, plant and animal extracts are available from, for example, Pan Laboratories (Bothell, WA) or MycoSearch (Durham, NC). Libraries of candidate compounds that can be screened using the methods of the present invention may be either prepared or purchased from a number of companies. Synthetic compound libraries are commercially available from, for example, Comgenex (Princeton, NJ), Brandon Associates (Merrimack, NH), Microsource (New Milford, CT), and Aldrich (Milwaukee, WI). Libraries of candidate compounds have also been developed by and are commercially available from large chemical companies, including, for example, Merck, Glaxo Wellcome, Bristol-Meyers-Squibb, Novartis, Monsanto/Searle, and Pharmacia UpJohn. Additionally, natural collections, synthetically produced libraries and compounds are readily modified through conventional chemical, physical, and biochemical means.

[0084] Useful regulators of iron metabolism and of hepcidin expression may be found within numerous classes of chemicals, including heterocycles, peptides, saccharides, steroids, and the like. In certain embodiments, the screening methods of the invention are used for identifying compounds or agents that are small molecules (*i.e.*, compounds or agents with a molecular weight < 600-700).

[0085] The screening of libraries according to the inventive methods will provide "hits" or "leads", *i.e.*, compounds that possess a desired but not-optimized biological activity. The next step in the development of useful drug candidates is usually the analysis of the relationship between the chemical structure of a hit compound and its biological or pharmacological activity. Molecular structure and biological activity are correlated by observing the results of systemic structural modification on defined biological endpoints. Structure-activity relationship information available from the first round of screening can then be used to generate small secondary libraries which are subsequently screened for compounds with higher affinity. The process of performing synthetic modifications of a biologically active compound to fulfill stereoelectronic, physicochemical, pharmacokinetic, and toxicologic factors required for clinical usefulness is called lead optimization.

[0086] The candidate compounds identified by the screening methods of the invention can similarly be subjected to a structure-activity relationship analysis, and chemically modified to provide improved drug candidates. The present invention also encompasses these improved drug candidates.

C - Identification of Regulators of Iron Metabolism and Regulators of Hepcidin Expression

[0087] According to the screening methods of the present invention, determination of the ability of a candidate compound to regulate iron metabolism or to regulate hepcidin expression includes comparison of at least one factor that is representative of the signaling activity of a TGF- β superfamily member measured in the test and control systems.

[0088] In the inventive screening methods, a candidate compound is identified as a regulator of hepcidin expression or activity and/or as a regulator of iron metabolism, if the factor measured in the test system is less or greater than the factor measured in the control system. More specifically, if a candidate compound is found to be an agonist of TGF- β or an antagonist of BMP, it is identified as an inhibitor of hepcidin expression or activity and/or as an enhancer of iron metabolism. Alternatively, if a candidate compound is found to be an antagonist of TGF- β or an agonist of BMP, it is identified as an enhancer of hepcidin expression or activity and/or as an inhibitor of iron metabolism.

[0089] Factors representative of the signaling activity of a TGF- β superfamily member include reporter assay signaling, and target gene expression (e.g., extracellular matrix protein genes). Other factors representative of the signaling activity of a TGF- β superfamily member include Smad phosphorylation, translocation of phosphorylated Smad proteins to the nucleus, and alterations in cell growth rates. In certain embodiments, the factor measured in the screening methods of

the invention is the amount of iron present in the system. In other embodiments, the factor measured is the level of hepcidin mRNA expression in the system. In still other embodiments, the factor measured is the hepcidin/actin ratio in the system.

[0090] Reproducibility of the results obtained in the inventive screening methods may be tested by performing the analysis more than once with the same concentration of the same candidate compound (for example, by incubating cells in more than one well of an assay plate). Additionally, since candidate compounds may be effective at varying concentrations depending on the nature of the compound and the nature of its mechanism(s) of action, varying concentrations of the candidate compound may be tested (for example, different concentrations can be added to different wells containing cells). Generally, candidate compound concentrations from 1 fM to about 10 mM are used for screening. Preferred screening concentrations are generally between about 10 pM and about 100 μ M.

[0091] In certain embodiments, the methods of the invention further involve the use of one or more negative and/or positive control compounds. A positive control compound may be any molecule or agent that is known to modulate the signaling activity of the TGF- β family member studied in the screening assay. A negative control compound may be any molecule or agent that is known to have no effect on the signaling activity of the TGF- β family member studied in the screening assay. In these embodiments, the inventive methods further comprise comparing the modulating effects of the candidate compound to the modulating effects (or absence thereof) of the positive or negative control compound. For example, Noggin and decorin may be used as positive controls for the inhibition of BMP signaling and TGF- β signaling, respectively.

D - Characterization of Candidate Compounds

[0092] As will be appreciated by those skilled in the art, it is generally desirable to further characterize regulators identified by the inventive screening methods.

[0093] For example, if a candidate compound has been identified as a modulator of the signaling activity in a given TGF- β superfamily member in a given cell culture system (e.g., an established cell line), it may be desirable to test this ability in a different cell culture system (e.g., primary or secondary cells). Alternatively or additionally, it may be desirable to directly evaluate the effects of the candidate compound on hepcidin expression, for example by quantitating hepcidin mRNA expression using real-time quantitative RT-PCR (as described in Example 1). It may also be desirable to evaluate the specificity of the candidate compound by testing its ability to modulate the signaling activity of other members of the TGF- β superfamily members. It may also be desirable to perform pharmacokinetics and toxicology studies.

[0094] Candidate compounds identified by screening methods of the invention may also be further tested in assays that allow for the determination of the compounds' *properties in vivo*. Suitable animal models include animal models that can exhibit iron deficient or iron overload or that have been determined to exhibit up-regulation of hepcidin expression or down-regulation of hepcidin expression.

[0095] Examples of animal models for iron overload include, but are not limited to, mice treated with carbonyl iron, β_2 -microglobulin knockout mice (C. Pigeon et al., J. Biol. Chem., 2001, 276: 7811-7819), USF2 (Upstream Stimulatory Factor 2) knockout mice (G. Nicolas et al., Proc. Natl. Acad. Sci. USA, 2001, 98: 8780-8785), and HFE knockout mice (K.A. Ahmad et al., Blood Cells Mol. Dis., 2002, 29: 361-366). Examples of animal models for iron deficiency include, but are not limited to, models of anemia in mice with acute hemolysis, provoked by phenylhydrazine, and mice with bleeding provoked by repeated phlebotomies (G. Nicolas et al., J. Clin. Invest., 2002, 110: 1037-1044). Examples of animal models exhibiting increased hepcidin mRNA expression include mice treated by partial hepatectomy (N. Kelley-Loughnane et al., Hepatology, 2002, 35: 525-534), by lipopolysaccharide (G.R. Lee, Semin. Hematol., 1983, 20: 61-80), and turpentine (G. Nicolas et al., J. Clin. Invest., 2002, 110: 1037-1044).

E - Pharmaceutical Compositions of Identified Regulators

[0096] The present invention also provides pharmaceutical compositions, which comprise, as active ingredient, an effective amount of at least one regulator of iron metabolism or at least one regulator of hepcidin expression or activity identified by an inventive screening assay. The pharmaceutical compositions of the invention may be formulated using conventional methods well known in the art. Such compositions include, in addition to the active ingredient(s), at least one pharmaceutically acceptable liquid, semiliquid or solid diluent acting as pharmaceutical vehicle, excipient or medium, and termed here "pharmaceutically acceptable carrier".

[0097] According to the present invention, pharmaceutical compositions may include one or more regulators of the invention as active ingredients. Alternatively, a pharmaceutical composition containing an effective amount of one inventive regulator may be administered to a patient in combination with or sequentially with a pharmaceutical composition containing a different inventive regulator. However, in both cases, the regulators preferably have the same regulatory effect on iron metabolism and/or hepcidin expression or activity. For example, an agonist of BMP and an antagonist of TGF- β , which both enhance hepcidin expression and inhibit iron metabolism, may be administered to a subject in a

single pharmaceutical composition, or in two different pharmaceutical compositions.

[0098] As will be appreciated by one skilled in the art, a regulator of hepcidin expression or an iron metabolism regulator, or a pharmaceutical composition thereof, may be administered serially or in combination with conventional therapeutics used in the treatment of iron metabolism disorders. Such therapeutics include iron supplements (in the case of diseases associated with iron deficiency) and iron chelating agents (in the case of diseases associated with iron overload). Iron supplements include, but are not limited to, ferrous fumarate, ferrous gluconate, ferrous sulfate, iron dextran, iron polysaccharide, iron sorbitol, sodium ferric gluconate, and iron sucrose. Iron chelating agents include, for example, desferrioxamine, bathophenanthroline, and Clioquinol. Iron supplements or iron chelating agents may be included in pharmaceutical compositions of the present invention. Alternatively, they may be administered in separate pharmaceutical compositions.

[0099] Alternatively or additionally, a regulator of hepcidin expression or an iron metabolism regulator, or a pharmaceutical composition thereof, may be administered serially or in combination with conventional therapeutic regimens for the treatment of iron metabolism disorders. These include, for example, phlebotomy, in the case of conditions associated with iron overload.

III. Methods of Treatment

[0100] In another aspect, the present invention provides methods for the treatment and/or prevention of conditions associated with perturbations in iron metabolism, including conditions associated with iron overload and conditions associated with iron deficiency. These methods comprise administering to a subject having or at risk or having such a condition, an effective amount of a compound that modulates the signaling activity of at least one TGF- β superfamily member, wherein modulation of the signaling activity of the TGF- β superfamily member results in regulation of hepcidin expression or activity in the subject.

[0101] The compound may be a known agonist or antagonist of the TGF- β superfamily member. Alternatively, the compound may be a regulator of iron metabolism or a regulator of hepcidin expression identified, for example, by a screening method provided by the present invention.

A - Iron Metabolism Diseases

[0102] Conditions that may be treated and/or prevented using the methods of the present invention include any disease, disorder, or syndrome associated with perturbations in iron metabolism. Perturbations in iron metabolism may be associated with disturbances in one or more of iron uptake, iron absorption, iron transport, iron storage, iron processing, iron mobilization, and iron utilization. Generally, perturbations in iron metabolism result in iron overload or iron deficiency.

[0103] Conditions associated with iron overload include both primary and secondary iron overload diseases, syndromes or disorders, including, but not limited to, hereditary hemochromatosis, porphyria cutanea tarda, hereditary spherocytosis, hypochromic anemia, hysererythropoietic anemia (CDAI), faciogenital dysplasia (FGDY), Aarskog syndrome, atransferrinemia, sideroblastic anemia (SA), pyridoxine-responsive sidero-blastic anemia, and hemoglobinopathies such as thalassemia and sickle cell. Some studies have suggested an association between iron metabolism disorders, such as thalassemia and hemochromatosis, and a number of disease states, such as type II (non-insulin dependent) diabetes mellitus and atherosclerosis (A.J. Matthews et al., J. Surg. Res., 1997, 73: 35-40; T.P. Tuomainen et al., Diabetes Care, 1997, 20: 426-428).

[0104] Diseases associated with iron deficiency include, but are not limited to, anemia of chronic disease, iron deficiency anemias, functional iron deficiency, and microcytic anemia. The term "**anemia of chronic disease**" refers to any anemia that develops as a result of, for example, extended infection, inflammation, neoplastic disorders, etc. The anemia which develops is often characterized by a shortened red blood cell life span and sequestration of iron in macrophages, which results in a decrease in the amount of iron available to make new red blood cells. Conditions associated with anemia of chronic disease include, but are not limited to, chronic bacterial endocarditis, osteomyelitis, rheumatic fever, ulcerative colitis, and neoplastic disorders. Further conditions include other diseases and disorders associated with infection, inflammation, and neoplasms, including, for example, inflammatory infections (e.g., pulmonary abscess, tuberculosis, etc), inflammatory noninfectious disorders (e.g., rheumatoid arthritis, systemic lupus erythrematosus, Crohn's disease, hepatitis, inflammatory bowel disease, etc.), and various cancers, tumors, and malignancies (e.g., carcinoma, sarcoma, lymphoma, etc.). Iron deficiency anemia may result from conditions such as pregnancy, menstruation, infancy and childhood, blood loss due to injury, etc.

[0105] It has also been suggested that iron metabolism plays a role in a number of other diseases states, including cardiovascular disease, Alzheimer's disease, Parkinson's disease, and certain types of colo-rectal cancers (see, for example, P. Tuomainen et al., Circulation, 1997, 97: 1461-1466; J.M. McCord, Circulation, 1991, 83: 1112-1114; J.L. Sullivan, J. Clin. Epidemiol., 1996, 49: 1345-1352; M.A. Smith et al., Proc. Nat. Acad. Sci., 1997, 94: 9866-9868; P. Riederer et al., J. Neurochem, 1989, 512: 515-520; P. Knekt et al., Int. J. Cancer, 1994, 56: 379-382).

B - Subject Selection

[0106] Subjects suitable to receive a treatment according to the inventive methods include individuals that have been diagnosed with a condition associated with perturbations in iron metabolism, including, but not limited to, the diseases and disorders listed above, and individuals that are susceptible to conditions associated with perturbations in iron metabolism. Suitable subjects may or may not have previously received traditional treatment for the condition.

[0107] Other suitable subjects are individuals that exhibit iron deficiency or iron overload. Iron overload and iron deficiency may be detected using a number of laboratory tests available in the art that allow for the determination of total iron-binding capacity (TIBC), levels of serum iron, ferritin, hemoglobin, hematocrit, and urinary creatinine.

C - Administration

[0108] A treatment according to methods of the present invention may consist of a single dose or a plurality of doses over a period of time. A regulator of hepcidin expression or modulator of iron metabolism, or a pharmaceutical composition thereof, may also be released from a depot form per treatment. The administration may be carried out in any convenient manner such as by injection (subcutaneous, intravenous, intramuscular, intraperitoneal, or the like), oral administration, or sublingual administration.

[0109] Effective dosages and administration regimens can be readily determined by good medical practice and the clinical condition of the individual patient. The frequency of administration will depend on the pharmacokinetic parameters of the compound and the route of administration. The optimal pharmaceutical formulation can be determined depending upon the route of administration and desired dosage. Such formulations may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the administered compounds.

[0110] Depending on the route of administration, a suitable dose may be calculated according to body weight, body surface area, or organ size. Optimization of the appropriate dosage can readily be made by those skilled in the art in light of pharmacokinetic data observed in human clinical trials. The final dosage regimen will be determined by the attending physician, considering various factors which modify the action of drugs, *e.g.*, the drug's specific activity, the severity of the damage and the responsiveness of the patient, the age, condition, body weight, sex and diet of the patient, the severity of any present infection, time of administration and other clinical factors. As studies are conducted, further information will emerge regarding the appropriate dosage levels and duration of treatment for various conditions associated with iron overload and iron deficiency.

Examples

[0111] The following examples describe some of the preferred modes of making and practicing the present invention. However, it should be understood that these examples are for illustrative purposes only and are not meant to limit the scope of the invention. Furthermore, unless the description in an Example is presented in the past tense, the text, like the rest of the specification, is not intended to suggest that experiments were actually performed or data were actually obtained.

[0112] Some of the results presented in this section have been described by the Applicants in a recent scientific manuscript (J.L. Babitt et al., "Bone Morphogenetic Protein Signaling by Hemojuvelin Regulates Hepcidin Expression", submitted to Nature Genetics on February 3, 2006). This manuscript is incorporated herein by reference in its entirety.

EXAMPLE 1: Effects of BMP and TGF- β on Hepcidin Transcription in Liver Cells

[0113] *Study Protocol.* The effects of BMP-2, Noggin (a well-known BMP inhibitor), and TGF- β 1 on hepcidin mRNA expression in HepG2 liver hepatoma cells were studied and quantitated using real-time quantitative RT-PCR.

[0114] HepG2 cells (ATTC Number HB-8065) were grown in α -MEM (Minimal Essential Medium Alpha Medium with L-Glutamine supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μ g/mL streptomycin) to 60% confluence on 6 cm tissue culture plates. Cells were then incubated in low-serum conditions (α -MEM with 1% FBS), with 1 μ g/mL Noggin.Fc (R & D Systems, Minneapolis, MN) at 37°C for 48 hours, or serum-starved for 6 hours followed by incubation with 50 ng/mL BMP-2 (R & D Systems) at 37°C for 16 hours, or with 1 ng/mL TGF- β 1 (R & D Systems) at 37°C for 16 hours. Alternatively, cells were incubated at 37°C for 72 hours with non-transferrin-bound iron (65 μ M Fe-NTA). Fe-NTA was generated by combining 1:1 molar ratio of FeCl₃ hexahydrate (Sigma) in 0.1 molar HCl with nitrilotriacetic acid (NTA, Sigma) in α -MEM medium supplemented with 20 mM Hepes pH 7.5, as previously described (E.W. Randell et al., J. Biol. Chem., 1994, 269: 16046-16053; S.G. Gehrke et al., Blood, 2003, 102: 371-376).

[0115] Total RNA was isolated from HepG2 cells treated as described above using the RNeasy Mini Kit (Qiagen, Valencia, CA) including DNase digestion with the RNase-free DNase Set (Qiagen), according to the manufacturer's instructions. Real-time quantification of mRNA transcripts was performed using a 2-step reverse transcriptase polymerase

chain reaction (RT-PCR) using the ABI Prism 7900HT Sequence Detection System and SDS software version 2.0. First strand cDNA synthesis was performed using iScript cDNA Synthesis Kit (Biorad Laboratories, Hercules, CA) according to the manufacturer's instructions using 2 μ g of total RNA template per sample. In a second step, transcripts of hepcidin were amplified with sense primer HepcF (5'-CTGCAACCCCAGGACAGAG-3') and antisense primer HepcR (5'-GGAATAAATAAGGAAGGGAGGGG-3') and detected using iTaq SYBR Green Supermix with ROX (Biorad). In parallel, transcripts of β -actin were amplified with sense primer BactF (5'-AGGATGCAGAAGGAGATCACTG-3') and antisense primer BactR (5'-GGGTGTAACGCAACTAAGTCATAG-3') and detected in a similar manner to serve as an internal control.

[0116] Standard curves for hepcidin and β -actin were generated from accurately determined dilutions of cDNA clones of hepcidin (IMAGE clones 4715540) and β -actin (IMAGE clone 3451917) as templates (IMAGE clones were purchased from Open Biosystems, and DNA sequenced to verify their inserts). Samples were analyzed in triplicate, and results are reported as the ratio of mean values for hepcidin to β -actin.

[0117] Results. As shown in Figure 1, incubation of HepG2 cells with Fe-NTA decreased the hepcidin/actin ratio expression approximately 6-fold (bar 4), a result which correlates well with a previously reported decrease in hepcidin expression caused by Fe-NTA in HepG2 cells (S.G. Gehrke et al., Blood, 2003, 102: 371-376). Significantly, incubation with 50 ng/mL BMP-2 increased the hepcidin/actin ratio expression by 10-fold ($\pm$ 8%) over baseline (compare bar 2 with bar 1). In contrast, incubation with 1 μ g/mL Noggin.Fc (which inhibits endogenous BMP signaling) decreased hepcidin/actin ratio expression 50-fold ($\pm$ 19%) below baseline (compare bar 3 with bar 1).

[0118] As shown in Figure 2, incubation of HepG2 cells with 50 ng/mL BMP-2 increased the hepcidin/actin ratio expression by 15-fold ($\pm$ 8%) over baseline (compare bar 2 with bar 1). In contrast, incubation with 1 μ g/mL Noggin.Fc (which inhibits endogenous BMP signaling) decreased hepcidin/actin ratio expression 6-fold ($\pm$ 23%) below baseline (compare bar 3 with bar 1). In addition, incubation with 1 ng/ml TGF- β 1 decreased hepcidin/actin ratio expression 6-fold ($\pm$ 6%) below baseline (compare bar 4 with bar 1).

EXAMPLE 2: HJV.Fc Protein as Modulator of Hepcidin Expression

[0119] Juvenile hemochromatosis is a severe variant of hemochromatosis caused by mutations in two genes that give indistinguishable phenotypes. One gene encodes hepcidin (HAMP, 19q13.1). The second gene has recently been identified as hemojuvelin (HJV, 1q21). Although the function of HJV is unknown, hepcidin levels are depressed in persons with HJV mutations, indicating that HJV may be a modulator of hepcidin expression. As already mentioned herein, HJV is also a member of the repulsive guidance molecule (RGM) family of proteins, including RGMa and DRAGON, neuronal adhesion molecules which were recently shown by the present Applicants to function as a BMP co-receptor (J.L. Babitt et al., J. Biol. Chem., 2005, 280: 29820-29827; T.A. Samad et al., J. Biol. Chem., 2005, 280: 14122-14129, each of which is incorporated herein by reference in its entirety). The study presented below was undertaken to investigate whether HJV could similarly mediate BMP signaling.

Materials and Methods

[0120] cDNA subcloning, cDNA encoding mutant murine HJV with a glycine to valine substitution at amino acid 313 (mHJV313V) was generated by an overlapping PCR strategy. Two primers 5'-ACCGAATTCGGGGACCTGGCTGGATAG-3' and 5'-CGGAGGGCATACCCCAACACACAG-3' were used to generate an N-terminal fragment of mHJV incorporating a substitution of valine for glycine at amino acid 313. Primers 5'-CTGTGTGTTGGGGATGCCCTCCG-3' and 5'-CCCTCTAGATGGTGCCAGTCTCCAAAAGC-3' were used to generate a C-terminal fragment of HJV with the identical substitution. A final round of PCR was performed using the outside primers to generate mutant mHJV313V, which was then subcloned into the expression vector pCDNA 3.1 (Invitrogen, Carlsbad, CA).

[0121] cDNA encoding soluble mHJV.Fc fusion protein was generated by PCR of the extracellular domains of wild-type murine HJV using primers: 5'-GGAAGCTTATGGGCCAGTCCCCTAGT-3' and 5'-CCGGATCCGCTAAGT-TCTCTAAATCCGTC-3', followed by subcloning into the mammalian expression vector pIgplus (R & D Systems, Minneapolis, MN) in-frame with the Fc portion of human IgG.

[0122] cDNA encoding flag-tagged human HJV (hHJV) was generated from human HJV transcript variant B (IMAGE clone 6198223), which does not contain exon 2, purchased from ATCC (# 10642497). Exon 2 which codes for the signal peptide of the full length HJV isoform (variant A, ACCESSION # NM213653) was amplified by PCR from human genomic DNA using the forward primer: 5'-CGAGAATTCACCTACAGGGCTTCCGGTCA-3' and the reverse primer: 5'-GCATTGAGAATGAGCATGTCCACAGAGGAGCAGCAG-3'. A downstream cDNA fragment corresponding to the rest of the coding sequence (including the stop codon) was amplified by PCR from the IMAGE clone using the forward primer 5'-CCTCTGTGGACATGCTCATTCTCAATGCAAGATCCTCCGCTG-3' and 5'-CGTCTCGAGTTACTGAATGCAAAGCCACAGAACAAAGAGC-3', as reverse primer. The two overlapping fragments were fused together by PCR and the full length cDNA product was then cut with EcoRI and XhoI and clone into pCDNA3.1 (Invitrogen), to generate

pCDNA3.1-hHJV. To generate N-terminal Flag-tagged hHJV, an upstream fragment corresponding to the beginning of exon 3 was generated by PCR using forward primer: 5'-GACAGATCTGCGGCCGCTCATTCTCAATGCAAGATCCTC-CG-3', and reverse primer: 5'-GAGCAGTTGTGCTGGATCATCAGG-3'. Following a Not I / Sac II digestion, the fragment was ligated together with a downstream Sac II/Xba I hHJV fragment, removed from pCDNA3.1-hHJV, into Not I / Xba I sites of p3FLAGCMV9 (Sigma).

[0123] cDNA encoding mutant Flag-tagged hHJV G99V (hG99V), with a valine to glycine substitution at amino acid 99, was generated from hHJV by site directed mutagenesis using the QuikChange kit (Stratagene, La Jolla, CA). cDNA encoding the hepcidin promoter luciferase construct was generated by subcloning the -2649 to +45 region of the human hepcidin promoter as in the pGL2-Basic vector (Promega, Madison, WI) upstream of the firefly luciferase reporter gene.

All cDNA's were sequenced to verify the fidelity of the constructs (MGH, Molecular Biology DNA Sequencing Core Facility).

[0124] Cell culture and transfection. CHO cells (American Type Culture Collection ATCC #CCL-61) were cultured in F-12K Nutrient Mixture, Kaighn's Modification (Invitrogen) supplemented with 10% fetal bovine serum (FBS) (Atlanta Biologicals, Lawrenceville, GA). HepG2 cells and Hep3B cells (ATCC #HB-8065 and #HB-8064) were cultured in Minimal Essential Alpha Medium with L-glutamine (α -MEM, Invitrogen) containing 10% FBS. HEK 293 cells (ATCC #CRL-1573) were cultured in Dulbecco's modification of Eagle's medium (DMEM; Cellgro Mediatech, Herndon, VA) supplemented with 10% FBS. All plasmid transfections were performed with Lipofectamine 2000 (Invitrogen) or Effectene transfection reagent (QIAGEN Inc, Valencia, CA) according to manufacturer instructions. Stably transfected cells were selected and cultured in 1 mg/ml Geneticin (Cellgro Mediatech, Herndon, VA).

[0125] Luciferase assay. HepG2 or Hep3B cells were transiently transfected with 2.5 μ g BMP responsive luciferase reporter (BRE-Luc), 2.5 μ g TGF- β responsive luciferase reporter, (CAGA)₁₂MPL-Luc (CAGA-Luc) (both kindly provided by Peter ten Dijke, Leiden University Medical Center, The Netherlands), or 2.5 μ g hepcidin promoter luciferase reporter construct, in combination with 0.25 μ g pRL-TK Renilla luciferase vector (Promega) to control for transfection efficiency, with or without co-transfection with wild-type or mutant HJV cDNA. Forty-eight hours after transfection, cells were serum starved in α -MEM supplemented with 1% FBS for 6 hours and treated with varying amounts of TGF- β 1 or BMP ligands (R & D Systems) for 16 hours, in the absence or presence of 1 μ g/ml noggin (R & D Systems) or 20 μ g/ml neutralizing anti-BMP-2/4 antibody (R & D Systems). Cells were lysed, and luciferase activity was determined with the Dual Reporter Assay according to the manufacturer's instructions (Promega). Experiments were performed in duplicate or triplicate wells. Relative luciferase activity was calculated as the ratio of firefly (reporter) and Renilla (transfection control) luciferase values, and is expressed as the fold increase over unstimulated cells transfected with reporter alone.

[0126] Purification of mHJV.Fc. CHO cells stably expressing mHJV.Fc were cultured in F-12K Nutrient Mixture, Kaighn's Modification, supplemented with 5% ultra-low IgG FBS (Invitrogen) using 175-cm² multifloor flasks (Denville Scientific, Southplainfield, NJ). mHJV.Fc was purified from the media of stably transfected cells via one-step Protein A affinity chromatography using HiTrap rProtein A FF columns (Amersham Biosciences, Piscataway, NJ) as previously described (E. del Re et al., J. Biol. Chem., 2004, 279: 22765-22772, which is incorporated herein by reference in its entirety). Purified protein was eluted with 100 mM glycine-HCl, pH 3.2 and neutralized with 0.3 M Tris-HCl pH 9 as previously described (E. del Re et al., J. Biol. Chem., 2004, 279: 22765-22772). mHJV.Fc was subjected to reducing sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and gels were stained with Bio-safe Coomassie blue (Bio-Rad, Hercules, CA) to determine purity and quantify protein concentration.

[0127] Generation of HJV antibody and immunoblot analysis. An affinity purified rabbit polyclonal anti-murine HJV antibody (α HJV) was raised against the peptide RVAEDVARAFSAEQDLQLC, amino acids 292-310 in the C-terminus of murine HJV upstream of its hydrophobic tail (G. Papamokolaou et al., Nat. Genet., 2004, 36: 77-82). Livers from 129S6/SvEvTac wild-type or *Hjv*^{-/-} mice (F.W. Huang et al., J. Clin. Invest., 2005, 115: 2187-2191), or cells transfected with wild-type or mutant HJV, were homogenized/sonicated in lysis buffer (200 mM Tris-HCl, pH 8, 100 mM NaCl, 1 mM EDTA, 0.5% NP-40, and 10% glycerol) containing a mixture of protease inhibitors (Roche, Mannheim, Germany) as previously described (J.L. Babitt et al., J. Biol. Chem., 2005, 280: 29820-29827, which is incorporated herein by reference in its entirety). For assays examining phosphorylated Smad expression, 1 mM sodium orthovanadate (Sigma, St. Louis, MO) and 1 mM sodium fluoride (Sigma) were added to the lysis buffer as phosphatase inhibitors. Purified mHJV.Fc, transfected cell lysates, or liver lysates, were subjected to reducing sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot as previously described (J.L. Babitt et al., J. Biol. Chem., 2005, 280: 29820-29827) using HJV antibody (1:1000, 4 mg/mL) at 4°C overnight, goat anti-human Fc antibody (1:1000) (Jackson ImmunoResearch Laboratories, West Grove, PA) at room temperature for 1 hour, or rabbit polyclonal anti-phosphosmad1/5/8 antibody (1:1000) (Cell Signaling, Beverly, MA) at 4°C overnight. Blots were stripped and re-probed with mouse monoclonal anti- β -actin antibody (1:5000) (clone AC 15, Sigma), rabbit polyclonal anti-Smad1 antibody (1:250) (Upstate Biotechnology, Lake Placid, NY) at 4°C overnight, or rabbit polyclonal anti-actin antibody (1:50) (Bio-medical Technologies, Inc., Stoughton, MA) for room temperature at 1 hour as loading controls.

[0128] HJV antibody recognizes major bands at ~49 kDa and ~30 kDa in the liver (lane 2, Fig. 3(A)), corresponding to the predicted size of full-length HJV and HJV which has been cleaved at a previously described proteolytic cleavage site. The ~62 kDa band likely represents a higher order form. No bands were seen after preincubation of antibody with

competing peptide (lane 1). Similar results were seen in transfected CHO cells with the difference in size likely due to differential glycosylation or altered processing (Fig. 3(B)). HJV.Fc cDNA, generated by fusing the extracellular domain of HJV with human Fc, was stably transfected into CHO cells, and HJV.Fc protein was purified from the media by one-step protein A chromatography. Western Blot of purified protein with anti-HJV antibody (lanes 1-2) or anti-Fc antibody (lanes 3-4) confirmed the presence of both domains in the purified protein and provided further validation of the anti-HJV antibody. Both antibodies recognized ~70-75 kDa and ~60 kDa bands, corresponding to the predicted sizes of the full-length and proteolytically cleaved proteins. A lower band at ~40-45 kDa suggests another possible proteolytic cleavage site.

[0129] Ligand iodination and crosslinking. Two (2) μ g of carrier-free human BMP-2 or BMP-4 ligand (R & D Systems) per reaction was iodinated with [^{125} I] by the modified chloramine-T method as previously described (C.A. Frolick et al., J. Biol. Chem., 1984, 259: 10995-11000, which is incorporated herein by reference in its entirety). [^{125} I]-BMP-2 was incubated with 60 ng mHJV.Fc or ALK5.Fc (R & D Systems) in 20 mM HEPES (pH 7.8) with 0.1% BSA and a mixture of protease inhibitors (Roche Diagnostics) or with buffer alone. This mixture was incubated in the absence or presence of 2.5 M disuccinimidyl suberate (DSS, Sigma, St. Louis, MO) followed by incubated with Protein A Sepharose beads (Amersham) as previously described (J.L. Babitt et al., J. Biol. Chem., 2005, 280: 29820-29827). Beads were washed with phosphate buffered saline (PBS) and protein eluted by nonreducing Laemmli sample buffer (Bio-Rad). Eluted protein was separated by SDS-PAGE and analyzed by autoradiography.

[0130] Quantitative reverse transcription polymerase chain reaction (RT-PCR). HepG2 or Hep3B cells were grown to 60% confluence on 6 cm tissue culture plates. Where indicated, cells were transfected with varying amounts of hHJV or hG99V cDNA. Twenty-four (24) hours after transfection, cells were serum-starved in α -MEM with 1% FBS followed by incubation with 50 ng/mL BMP-2 at 37°C for various times or with 1 μ g/mL noggin at 37°C for 48 hours. For cycloheximide experiments, 10 μ g/ml cycloheximide was added for 30 minutes prior to addition of BMP-2. Total RNA was isolated using the RNeasy Mini Kit (QIAGEN Inc., Valencia CA), including DNase digestion with the RNase-Free DNase Set (QIAGEN) according to the manufacturer's instructions. Real time quantification of mRNA transcripts was performed using a 2-step reverse transcriptase polymerase chain reaction (RT-PCR) using the ABI Prism 7900HT Sequence Detection System and SDS software version 2.0. First strand cDNA synthesis was performed using iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions using 2 μ g total RNA template per sample.

[0131] In a second step, transcripts of hepcidin were amplified with sense primer HepcF 5'-CTGCAACCCAGGACA-GAG-3' and antisense primer HepcR 5'-GGAATAAATAAGGAAGGGAGGGG-3' and detected using iTaq SYBR Green Supermix with ROX (Biorad) according to the manufacturer's instructions. In parallel, transcripts of β -actin were amplified with sense primer BactF 5'-AGGATGCAGAAGGAGATCACTG-3' and antisense primer 5'-GGGTGTAACG-CAACTAAGTCATAG-3' and detected in a similar manner to serve as an internal control. Standard curves for hepcidin and β -actin were generated from accurately determined dilutions of plasmids containing cDNA sequences of hepcidin and β -actin as templates (IMAGE clones 4715540 and 3451917 from Open Biosystems followed by sequence analyses to verify the proposed insert). Samples were analyzed in triplicate, and results are reported as the ratio of mean values for hepcidin to β -actin. Transcripts for BMP-2 and BMP-4 were amplified from HepG2 cDNA generated above using the forward primer 5'-CGTGACCAGACTTTTGGACAC-3' and reverse primer 5'-GGCATGATTAGTGGAGTTCAG-3' (for BMP-2) and the forward primer: 5'-AGCAGCCAACTATGGGCTA-3' and reverse primer 5'-TGGTTGAGTTGAGGT-GGTCA-3' (for BMP-4).

[0132] Primary Hepatocyte Isolation and Culture. Primary hepatocytes were isolated by collagenase digestion of livers from 8 to 10 week old 129S6/SvEvTac wild-type or *Hjv*^{-/-} mice (F.W. Huang et al., J. Clin. Invest., 2005, 115: 2187-2191) using previously described methods (J. Lin et al., Cell, 2004, 119: 121-135, which is incorporated herein by reference in its entirety). Briefly, mice were perfused through the inferior vena cava with calcium-free Hank's Balanced Salt Solution (HBSS) (Mediatech Inc.) supplemented with 0.5 mM EDTA and 16.7 mM sodium bicarbonate for 4 minutes at a rate of ~1.5 mL/min. Mice were subsequently perfused with calcium-containing HBSS containing 0.05% collagenase (Sigma), 1% bovine serum albumin and 16.7 mM sodium bicarbonate for 8 min. After enzymatic digestion, hepatocytes were liberated into culture medium [1:1 Dulbecco's modified Eagle's/Ham's F12 medium (Gibco™, Grand Island, NY) supplemented with 100 IU/ml penicillin, 100 μ g/ml streptomycin, 18 mM HEPES, 1 mM sodium pyruvate, 10 μ g/ml insulin, 5.5 μ g/ml transferrin, and 5 ng/ml selenium (ITS; Sigma), 2 mM L-glutamine, 0.1 mM non-essential amino acids (Gibco™), 10% FBS (HyClone, Logan UT)], passed through a 100 μ m BD Falcon™ mesh cell strainer (BD Biosciences, San Jose CA), centrifuged, gently washed with culture medium, and counted.

[0133] Cells (> 90% hepatocytes by microscopy) were seeded on collagen-coated plates (Sigma) at 5×10^5 cells/ 60 mm dish. After 2 to 3 hours, cells were washed with PBS, serum starved with culture medium containing 1% FBS for 6 hours, and stimulated with recombinant human BMP-2 at varying concentrations for 12 hours. RNA was isolated using the RNeasy kit according to manufacturer's directions (QIAGEN).

[0134] Northern Blot Analysis. Total RNA (2.5 μ g) from primary hepatocytes was separated on a 1% formaldehyde agarose gel and transferred onto Hybond N+ membranes (Amersham Pharmacia Biotech). Membranes were baked for two hours at 80°C under vacuum and hybridized with radioactively labeled probes specific for mouse hepcidin 1 amplified

from Soares mouse p3NMF19.5 Mus musculus cDNA IMAGE clone: 317863 with primers 5'-TCCTTAGACTGCACAG-CAGAA-3' and 5'-ATAAATAAGGACGGGAGGGG-3' and β -actin (S. Alonso et al., J. Mol. Evol., 1986, 23: 11-22). Expression was quantified using a phosphorimager (Molecular Dynamics, now Amersham Biosciences) and normalized to β -actin or 28S RNA as loading controls.

[0135] Statistical Analysis. A two-tailed Student's *t*-test was used with a P value of < 0.05 to determine statistical significance.

Result 1: HJV induces BMP but not TGF- β Signals

[0136] HepG2 cells were transfected with a BMP-responsive luciferase reporter (BRE-Luc, Figure 4, panels A and C) or TGF- β responsive luciferase reporter (CAGA-Luc, Figure 4, panel B) either alone or in combination with cDNA encoding HJV. Transfected cells were then incubated with or without 0.5 nM BMP-2, BMP-4, or 40 pM TGF- β 1 for 16 hours followed by measurement of luciferase activity. Stimulation with BMP or TGF- β increased the relative luciferase activity for their respective reporters compared with unstimulated cells (A and B, compare bars 2 to 1). Co-transfection with HJV similarly increased BRE luciferase activity even in the absence of exogenous BMP stimulation (A, bar 3). HJV-mediated BMP signaling was dose dependent (C, grey bars), and the presence of HJV augmented signaling produced by exogenous BMP (C, black bars). In contrast, co-transfection with HJV (up to 1 μ g) did not increase CAGA-luciferase activity above baseline (B, bar 3). Taken together, these results demonstrate that HJV can mediate BMP signaling but not TGF- β signaling, and that HJV behaves in a manner consistent with a possible accessory receptor for BMP-2,

Result 2: HJV Mediated BMP Signaling is Inhibited by Noggin

[0137] The ability of HJV to mediate BMP signaling even in the absence of exogenous BMP ligand raises the question of whether HJV is acting in a ligand-independent manner, or whether it is augmenting signaling by endogenous BMP ligands. Studies were therefore undertaken to determine whether HJV-mediated signaling could be inhibited by Noggin, a soluble inhibitor of BMP signaling that functions by binding to BMP ligands and blocking the binding epitopes for BMP receptors.

[0138] HepG2 cells were co-transfected with BRE-Luc and HJV cDNA or empty vector. Transfected cells were incubated with or without 0.5 nM exogenous BMP-2 in the presence or absence of 1 μ g Noggin protein for 16 hours followed by measurement of luciferase activity. The results obtained are reported in Figure 5.

[0139] In the absence of Noggin, co-transfection with HJV cDNA increased BRE luciferase activity 10 fold above baseline (compare bar 2 to bar 1). Similarly, incubation with exogenous BMP-2 increased BRE luciferase activity 12 fold over baseline (compare bar 4 to bar 1). This stimulation by either HJV or exogenous BMP could be blocked by the presence of Noggin protein (bars 3, 5). In contrast, Noggin did not affect TGF- β 1 induced CAGA luciferase activity (bars 6-8). This data suggests that HJV generates BMP signals in a ligand-dependent manner, presumably *via* endogenously expressed BMP ligands.

Result 3: HJV.Fc Binds BMP-2 Selectively

[0140] HJV.Fc was incubated overnight with ^{125} I-labeled BMP-2 with or without excess cold BMP-2, -4, -7 or TGF- β 1, followed by incubation on protein A coated plates and determination of radioactivity (Fig. 6(A) and Fig. 7). Alternatively, chemical crosslinking of HJV.Fc with ^{125} I-labeled BMP-2 was performed using DSS in a cell free system (Fig. 6(B)).

[0141] As shown in Fig. 6(A) HJV.Fc was able to bind to ^{125} I-BMP-2 in a dose dependent fashion. Binding of HJV.Fc to ^{125} I-BMP-2 was competitively inhibited by excess cold BMP-2 but not by BMP-4, BMP-7 or TGF- β 1 (see Fig 7). ^{125} I-BMP-2 can be chemically crosslinked with HJV.Fc in the presence of DSS (Fig 6(B), lane 4) and this can be inhibited by excess cold BMP-2 (lane 5). As negative controls, no band was seen in the absence of DSS (lanes 1 and 2) or when buffer alone (lane 3) or ALK5.Fc (a TGF- β type I receptor, lane 6) was used in place of HJV.Fc.

Result 4: HJV Mediated BMP Signaling is Inhibited by Dominant Negative Type I Receptors ALK3 and ALK6 and by Dominant Negative Smad1

[0142] HepG2 cells were co-transfected with BRE-Luc and HJV either alone or in combination with dominant negative BMP type I receptor ALK3 (ALK3 DN) or ALK6 (ALK6 DN) (Fig. 8(A)), or with wildtype (WT) versus dominant negative (DN) R-Smad 1 (Fig. 8(B)). Transfected cells were then incubated in the presence or absence of 0.5 nM BMP-2 for 16 hours followed by measurement of luciferase activity.

[0143] As shown in Fig. 8(A), transfection with HJV or incubation of cells with exogenous BMP-2 increased BRE luciferase activity above baseline ~15-20 fold (compare bars 2 and 5 to bar 1). This stimulation by either HJV or exogenous BMP-2 could be blocked by co-transfection with dominant negative ALK3 (bars 3, 6) or dominant negative ALK6 (bars 4, 7).

[0144] As shown in Fig 8(B), transfection with WT Smad 1 alone increased BRE luciferase activity ~12 fold above baseline (compare bars 2 to 1). In contrast, transfection with DN Smad 1 alone decreased BRE luciferase activity below baseline (compare bar 3 to bar 1). This provides further support that there is basal signal transduction *via* the BMP pathway in these cells in the absence of exogenously added ligand, and this signaling can be augmented by the presence of additional WT Smad 1 and inhibited by DN Smad 1. Transfection with HJV increased BRE luciferase activity ~12 fold above baseline (bar 4). Co-transfection of WT Smad 1 with HJV further augmented the signaling induced by either WT Smad 1 or HJV alone (compare bar 5 to 2, 4). Co-transfection of DN Smad 1 with HJV blocked the increase in signal seen with HJV alone (compare bar 6 to bar 4). Similar results were seen for the effect of WT Smad 1 and DN Smad 1 on exogenous BMP-2 stimulation (bars 7-9). Thus, HJV-mediated BMP signaling occurs *via* the classical BMP signaling pathway through BMP type I receptors ALK3 and ALK6 as well as R-Smad1.

Result 5: Production and Characterization of Mutant HJVG313V and HJVG313 V.Fc Fusion Protein

[0145] The most common mutation in HJV resulting in juvenile hemochromatosis is a point mutation substituting valine for glycine at amino acid 320 (corresponding to amino acid 313 in murine HJV). Mutant HJVG313V and soluble HJVG313V.Fc cDNA were made using PCR and subcloning techniques as described above, transfected into CHO cells, and analyzed by reducing SDS PAGE followed by Western blot with anti-HJV antibody (Fig. 9(A) and (B) left panel) or anti-Fc antibody (Fig. 9(B), right panel). Alternatively, unpermeabilized transfected cells were analyzed by immunofluorescence microscopy using anti-HJV antibody (Fig. 10).

[0146] As shown in Fig. 9(A), mutant HJVG313V is expressed in CHO cells, but migrates with a different pattern than wild-type HJV suggesting it is processed differently, at least in this cell type. Mutant HJVG313V.Fc also appears to be processed differently from wild-type HJV.Fc with a loss of the ~60kDa band (see Fig. 9(B)).

[0147] As shown in Fig. 10, both wildtype HJV and mutant HNG313V are expressed on the cell surface in a punctate distribution.

Result 6: Mutant HJVG313V decreases BMP Signaling Ability Compared to Wild-Type HJV

[0148] HepG2 cells were transfected with BRE-Luc alone or in combination with increasing concentrations of wildtype HJV or mutant HJVG313V cDNA. Transfected cells were incubated in the presence or absence of 0.5 nM BMP-2 for 16 hours followed by measurement of luciferase activity. As shown in Figure 11, in the absence of exogenous ligand, wildtype HJV increased BRE luciferase activity up to 23 fold over baseline. This stimulation was on the order of that seen with 0.5 nM exogenous BMP-2. In contrast, mutant HJVG313V increased BRE luciferase activity only to a maximum of 9 fold. This suggests that mutant HJVG313V, which in humans can result in juvenile hemochromatosis, has decreased BMP signaling ability in liver cells, raising the question of whether BMP signaling might play a role in iron metabolism.

Conclusions

[0149] As reported above, the Applicants have shown that (1) HJV induces BMP but not TGF- β signaling; (2) HJV signaling is blocked by Noggin, a well-known BMP inhibitor; (3) HJV binds directly to radiolabeled BMP-2 ligand; (4) HJV signals *via* the BMP type I receptors, ALK-3 and ALK-6; (5) HJV signals *via* the BMP R-Smad, Smad1; (6) an HJV mutant known to cause juvenile hemochromatosis decreases BMP signaling ability; and (7) BMP increases, while Noggin decreases, hepcidin expression in liver cells.

[0150] These results suggest that HJV is a novel BMP co-receptor whose BMP signaling ability is important in regulating iron metabolism. Mutations in HJV could lead to decreased BMP signaling in liver cells, which could then decrease hepcidin expression, thereby explaining why persons with HJV mutations have depressed hepcidin levels and thus iron overload. The present findings regarding the novel mechanism of action of HJV reveal a heretofore undiscovered link between BMP signaling and iron metabolism, and could lead to novel treatment strategies of disorders of iron metabolism such as hemochromatosis and anemia of chronic disease.

EXAMPLE 3: Effects of BMP-2 on Iron Binding Capacity *in vivo*

[0151] **Study Protocol.** Normal mice were injected intraorbitally with 18 μ g of BMP-2 (equivalent to 1 mg per kg body weight), or with carrier solution as a control. After 4 hours, blood was harvested and serum iron levels and total iron binding capacity was measured using colorimetric assays.

[0152] As shown on Figure 12, the injection of BMP-2 led to significant decreases in both the serum iron and the total iron binding capacity. This result indicates that BMP ligands and BMP inhibitors will be useful as therapeutic agents to regulate iron levels in whole animals including humans.

EXAMPLE 4: Proteolytically Stable HJV, RGMa, and Dragon Mutants

[0153] Experiments were undertaken to demonstrate the feasibility of producing mutant HJV, RGMa and Dragon proteins that, in contrast to their corresponding wild-type proteins, do not undergo proteolytic cleavage.

[0154] Mouse RGMa-D169A.Fc mutant cDNA was generated and expressed in HEK cell supernatants. Figure 13 shows that the purified protein obtained is not proteolytically cleaved compared to wild-type mouse RGMa.Fc protein.

[0155] Similarly, mouse Dragon-D171A.Fc mutant cDNA was generated and expressed in HEK cell supernatants. The purified mutant protein obtained was shown to be stable to proteolytic cleavage compared to wild-type mouse Dragon.Fc protein (see Figure 14).

[0156] In a third experiment, the human HJV-D172A mutant cDNA was generated and expressed in HEK cell supernatants. As shown on Figure 15, in contrast to the wild-type human HJV protein, the mutant HJV protein did not undergo proteolytic cleavage.

[0157] Mutant HJV, RGMa, and Dragon fusion proteins that are more stable to proteolytic cleavage than the wild-type versions could be advantageously used in the methods of the present invention.

Other Embodiments

[0158] Other embodiments of the invention will be apparent to those skilled in the art from a consideration of the specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope of the invention being indicated by the following claims.

ASPECTS OF THE INVENTION**[0159]**

1. A method for regulating hepcidin expression or activity in a subject, the method comprising administering to the subject an effective amount of a compound that modulates the signaling activity of at least one TGF- β superfamily member, thereby regulating hepcidin expression or activity in the subject.

2. The method of aspect 1, wherein the at least one TGF- β 1 superfamily member is TGF- β or BMP.

3. The method of aspect 2, wherein the compound comprises an agent selected from the group consisting of an agonist of TGF- β , an antagonist of TGF- β , an agonist of BMP, an antagonist of BMP, and combinations thereof.

4. The method of aspect 3, wherein the agent is selected from the group consisting of a HJV.Fc fusion protein, a Dragon.Fc fusion protein, a DLN.Fc fusion protein, a sT β R11.Fc fusion protein, a sT β R11-B.Fc fusion protein, and a sT β R11 Δ .Fc fusion protein.

5. The method of aspect 3, wherein the agent comprises a fusion protein selected from the group consisting of a mutant HJV.Fc fusion protein, a mutant Dragon.Fc fusion protein and mutant DLN.Fc fusion protein, wherein the fusion protein is non-proteolytically cleavable.

6. The method of aspect 2, wherein the subject has or is at risk of having a condition associated with iron deficiency.

7. The method of aspect 6, wherein the condition associated with iron deficiency is selected from the group consisting of anemia of chronic disease, iron deficiency anemia, functional iron deficiency, and microcytic anemia.

8. The method of aspect 2, wherein the subject has or is at risk of having a condition associated with iron overload.

9. The method of aspect 8, wherein the condition associated with iron overload is selected from the group consisting of adult hemochromatosis and juvenile hemochromatosis.

10. The method of aspect 2, wherein the subject has or is at risk of having a condition associated with iron overload and wherein regulating hepcidin expression or activity in the subject comprises enhancing hepcidin expression or activity in the subject.

11. The method of aspect 10, wherein the compound comprises an agent selected from the group consisting of an agonist of BMP, an antagonist of TGF- β , and combinations thereof.

12. The method of aspect 11, wherein the antagonist of TGF- β is selected from the group consisting of a sT β RII.Fc fusion protein, a sT β RII-B.Fc fusion protein, and a sT β RITIA.Fc fusion protein.

13. The method of aspect 10, wherein the condition associated with iron overload is selected from the group consisting of adult hemochemostasis and juvenile hemochemostasis.

14. The method of aspect 2, wherein the subject has or is at risk of having a condition associated with iron deficiency and wherein regulating hepcidin expression or activity in the subject comprises inhibiting hepcidin expression or activity in the subject.

15. The method of aspect 14, wherein the compound comprises an agent selected from the group consisting of an agonist of TGF- β , an antagonist of BMP, and combinations thereof.

16. The method of aspect 15, wherein the antagonist of BMP is selected from the group consisting of a HJV.Fc fusion protein, a Dragon.Fc fusion protein, and a DLN.Fc fusion protein.

17. The method of aspect 15, wherein the antagonist of BMP comprises a fusion protein selected from the group consisting of a mutant HJV.Fc fusion protein, a mutant Dragon.Fc fusion protein and mutant DLN.Fc fusion protein, wherein the fusion protein is non-proteolytically cleavable.

18. The method of aspect 10, wherein the condition associated with iron deficiency is selected from the group consisting of anemia of chronic disease, iron deficiency anemia, functional iron deficiency, and microcytic anemia.

19. A method for regulating hepcidin expression or activity in a biological system, the method comprising contacting the biological system with an effective amount of a compound that modulates the signaling activity of at least one TGF- β superfamily member, thereby regulating hepcidin expression or activity in the biological system.

20. The method of aspect 19, wherein the at least one TGF- β superfamily member is TGF- β or BMP.

21. The method of aspect 20, wherein the compound comprises an agent selected from the group consisting of an agonist of TGF- β , an antagonist of TGF- β , an agonist of BMP, an antagonist of BMP, and combinations thereof.

22. The method of aspect 21, wherein the agent is selected from the group consisting of a HJV.Fc fusion protein, a Dragon.Fc fusion protein, a DLN.Fc fusion protein, a sT β RII.Fc fusion protein, a sT β RII-B.Fc fusion protein, and a sT β RIIIA.Fc fusion protein.

23. The method of aspect 19, wherein the biological system is selected from the group consisting of a cell, a biological fluid, a biological tissue, and an animal.

24. The method of aspect 19, wherein the biological system exhibits iron overload, and wherein regulating hepcidin expression or activity in the biological system comprises enhancing hepcidin expression or activity in the biological system.

25. The method of aspect 24, wherein the compound comprises an agent selected from the group consisting of an agonist of BMP, an antagonist of TGF- β , and combinations thereof.

26. The method of aspect 25, wherein the antagonist of TGF- β is selected from the group consisting of a sT β RII.Fc fusion protein, a sT β RII-B.Fc fusion protein, and a sT β RITIA.Fc fusion protein.

27. The method of aspect 19, wherein the biological system exhibits iron deficiency, and wherein regulating hepcidin expression or activity in the biological system comprises inhibiting hepcidin expression or activity in the biological system.

28. The method of aspect 27, wherein the compound comprises an agent selected from the group consisting of an agonist of TGF- β , an antagonist of BMP, and combinations thereof.

29. The method of aspect 28, wherein the antagonist of BMP is selected from the group consisting of a HJV.Fc fusion protein, a Dragon.Fc fusion protein, and a DLN.Fc fusion protein.

30. The method of aspect 28, wherein the antagonist of BMP comprises a fusion protein selected from the group consisting of a mutant HJV.Fc fusion protein, a mutant Dragon.Fc fusion protein and mutant DLN.Fc fusion protein, wherein the fusion protein is non-proteolytically cleavable.

31. A method for regulating iron metabolism or an iron metabolic process in a subject, the method comprising administering to the subject an effective amount of a compound that modulates the signaling activity of at least one TGF- β superfamily member, thereby regulating iron metabolism or the iron metabolic process in the subject.

32. The method of aspect 31, wherein administering the compound results in regulation of hepcidin expression or activity in the subject.

33. The method of aspect 31, wherein the at least one TGF- β superfamily member is TGF- β or BMP.

34. The method of aspect 33, wherein the compound comprises an agent selected from the group consisting of an agonist of TGF- β , an antagonist of TGF- β , an agonist of BMP, an antagonist of BMP, and combinations thereof.

35. The method of aspect 34, wherein the agent is selected from the group consisting of a HJV .Fc fusion protein, a Dragon.Fc fusion protein, a DLN.Fc fusion protein, a sT β RILFc fusion protein, a sT β RIL-B.Fc fusion protein, and a sT β RIL Δ .Fc fusion protein.

36. The method of aspect 31, wherein the iron metabolic process is selected from the group consisting of iron uptake, iron absorption, iron transport, iron storage, iron processing, iron mobilization, and iron utilization.

37. The method of aspect 31 wherein the subject has or is at risk of having a condition associated with iron overload and wherein regulating iron metabolism or an iron metabolic process comprises inhibiting iron metabolism or an iron metabolic process in the subject.

38. The method of aspect 37, wherein the compound comprises an agent selected from the group consisting of an agonist of BMP, an antagonist of TGF- β , and combinations thereof.

39. The method of aspect 38, wherein the antagonist of TGF- β is selected from the group consisting of a sT β RIL.Fc fusion protein, a sT β RIL-B.Fc fusion protein, and a sT β RIL Δ .Fc fusion protein.

40. The method of aspect 37, wherein the condition associated with iron overload is selected from the group consisting of adult hemochromatosis and juvenile hemochromatosis.

41. The method of aspect 31, wherein the subject has or is at risk of having a condition associated with iron deficiency and wherein regulating iron metabolism or a iron metabolic process comprises enhancing iron metabolism or the iron metabolic process in the subject.

42. The method of aspect 41, wherein the compound comprises an agent selected from the group consisting of an agonist of TGF- β , an antagonist of BMP, and combinations thereof.

43. The method of aspect 42, wherein the antagonist of BMP is selected from the group consisting of a HJV.Fc fusion protein, a Dragon.Fc fusion protein, and a DLN.Fc fusion protein.

44. The method of aspect 42, wherein the antagonist of BMP comprises a fusion protein selected from the group consisting of a mutant HJV.Fc fusion protein, a mutant Dragon.Fc fusion protein and mutant DLN.Fc fusion protein, wherein the fusion protein is non-proteolytically cleavable.

45. The method of aspect 41, wherein the condition associated with iron deficiency is selected from the group consisting of anemia of chronic disease, iron deficiency anemia, functional iron deficiency, and microcytic anemia.

46. A method for regulating iron metabolism or an iron metabolic process in a biological system, the method comprising contacting the biological system with an effective amount of a compound that modulates the signaling activity of at least one TGF- β 1 superfamily member, thereby regulating iron metabolism or the iron metabolic process in the biological system.

47. The method of aspect 46, wherein the at least one TGF- β 1 superfamily member is TGF- β or BMP.

48. The method of aspect 47, wherein the compound comprises an agent selected from the group consisting of an agonist of TGF- β , an antagonist of TGF- β , an agonist of BMP, an antagonist of BMP, and combinations thereof.

49. The method of aspect 48, wherein the agent is selected from the group consisting of a HJV.Fc fusion protein, a Dragon.Fc fusion protein, a DLN.Fc fusion protein, a sT β RII.Fc fusion protein, a sT β RII-B.Fc fusion protein, and a sT β RIII Δ .Fc fusion protein.

50. The method of aspect 46, wherein the biological system is selected from the group consisting of a cell, a biological fluid, a biological tissue, and an animal.

51. The method of aspect 46, wherein the iron metabolic process is selected from the group consisting of iron uptake, iron absorption, iron transport, iron storage, iron processing, iron mobilization, and iron utilization.

52. The method of aspect 44, wherein the biological system exhibits iron overload, and wherein regulating iron metabolism or an iron metabolic process comprises inhibiting iron metabolism or the iron metabolic process in the biological system.

53. The method of aspect 52, wherein the compound comprises an agent selected from the group consisting of an agonist of BMP, an antagonist of TGF- β , and combinations thereof.

54. The method of aspect 53, wherein the antagonist of TGF- β is a sT β RII.Fc fusion protein, a sT β RII-B.Fc fusion protein, and a sT β RIII Δ .Fc fusion protein.

55. The method of aspect 43, wherein the biological system exhibits iron deficiency, and wherein regulating iron metabolism or an iron metabolic process comprises enhancing iron metabolism or the iron metabolic process in the biological system.

56. The method of aspect 55, wherein the compound comprises an agent selected from the group consisting of an agonist of TGF- β , an antagonist of BMP, and combinations thereof.

57. The method of aspect 56, wherein the antagonist of BMP is selected from the group consisting of a HJV .Fc fusion protein, a Dragon.Fc fusion protein, and a DLN.Fc fusion protein.

58. The method of aspect 56, wherein the antagonist of BMP comprises a fusion protein selected from the group consisting of a mutant HJV.Fc fusion protein, a mutant Dragon.Fc fusion protein and mutant DLN.Fc fusion protein, wherein the fusion protein is non-proteolytically cleavable.

59. A method for treating or preventing a condition associated with perturbations in iron metabolism or an iron metabolic process in a subject, the method comprising administering to the subject an effective amount of a compound that modulates the signaling activity of at least one TGF- β superfamily member, thereby treating or preventing the condition of the subject.

60. The method of aspect 59, wherein the iron metabolic process is selected from the group consisting of iron uptake, iron absorption, iron transport, iron storage, iron processing, iron mobilization, and iron utilization.

61. The method of aspect 60, wherein the at least one TGF- β superfamily member is TGF- β or BMP.

62. The method of aspect 59, wherein administering the compound results in regulation of hepcidin expression or activity in the subject.

63. The method of aspect 61, wherein the compound comprises an agent selected from the group consisting of an agonist of TGF- β , an antagonist of TGF- β , an agonist of BMP, an antagonist of BMP, and combinations thereof.

64. The method of aspect 63, wherein the agent is selected from the group consisting of a HJV .Fc fusion protein, a Dragon.Fc fusion protein, a DLN.Fc fusion protein, a sT β RII.Fc fusion protein, a sT β RII-B.Fc fusion protein, and

a sTβRIIIΔ.Fc fusion protein.

65. The method of aspect 59, wherein the subject has or is at risk of having a condition associated with iron deficiency, and the compound comprises an agent selected from the group consisting of an antagonist of BMP, an agonist of TGF-β, and combinations thereof.

66. The method of aspect 65, wherein the antagonist of BMP is selected from the group consisting of a HJV.Fc fusion protein, a Dragon.Fc fusion protein, and a DLN.Fc fusion protein.

67. The method of aspect 65, wherein the antagonist of BMP comprises a fusion protein selected from the group consisting of a mutant HJV.Fc fusion protein, a mutant Dragon.Fc fusion protein and mutant DLN.Fc fusion protein, wherein the fusion protein is non-proteolytically cleavable.

68. The method of aspect 65, wherein the condition associated with iron deficiency is selected from the group consisting of anemia of chronic disease, iron deficiency anemia, functional iron deficiency, and microcytic anemia.

69. The method of aspect 68, wherein the chronic disease is a disorder selected from the group consisting of an inflammation, an infection, an immunodeficiency disorder, and a neoplastic disorder.

70. The method of aspect 65, further comprising administering an iron supplementation treatment to the subject.

71. The method of aspect 70, wherein the iron supplementation treatment is selected from the group consisting of ferrous fumarate, ferrous gluconate, ferrous sulfate, iron dextran, iron polysaccharide, iron sorbitol, sodium ferric gluconate, and iron sucrose.

72. The method of aspect 59, wherein the subject has or is at risk of having a condition associated with iron overload, and the compound comprises an agent selected from the group consisting of an agonist of BMP, an antagonist of TGF-β, and combinations thereof.

73. The method of aspect 72, wherein the antagonist of TGF-β is selected from the group consisting of a sTβRII.Fc fusion protein, a sTβRII-B.Fc fusion protein, and a sTβRIIIΔ.Fc fusion protein

74. The method of aspect 72, wherein the condition associated with iron overload is selected from the group consisting of adult hemochromatosis and juvenile hemochromatosis.

75. The method of aspect 72, further comprising administering an iron chelation treatment to the subject.

76. The method of aspect 75, wherein the iron chelation treatment is selected from the group consisting of desferrioxamine, bathophenanthroline, and Clioquinol

77. The method of aspect 72, further comprising performing phlebotomy to the subject.

78. A method for identifying a compound that regulates hepcidin expression or activity in a system, wherein the system expresses at least one TGF-β superfamily member, the method comprising steps of: incubating the system with a candidate compound under conditions and for a time sufficient for the candidate compound to modulate the signaling activity of the at least one TGF-β superfamily member, to obtain a test system; measuring, in the test system, at least one factor that is representative of the signaling activity of the at least one TGF-β superfamily member; incubating the system under the same conditions and for the same time absent the candidate compound, to obtain a control system; measuring the factor in the control system; comparing the factor measured in the test and control systems; and determining that the candidate compound regulates hepcidin expression or activity in the system, if the factor measured in the test system is less than or greater than the factor measured in the control system.

79. The method of aspect 78, wherein the at least one TGF-β superfamily member is TGF-β or BMP.

80. The method of aspect 79, wherein the system is a cell, a biological fluid, a biological tissue, or an animal.

81. The method of aspect 78, wherein the system expresses TGF-β.

82. The method of aspect 81, wherein the candidate compound enhances the signaling activity of TGF- β and is identified as a compound that inhibits hepcidin expression or activity in the system.

83. The method of aspect 81, wherein the candidate compound inhibits the signaling activity of TGF- β and is identified as a compound that enhances hepcidin expression or activity in the system.

84. The method of aspect 78, wherein the system expresses BMP.

85. The method of aspect 84, wherein the candidate compound enhances the signaling activity of BMP and is identified as a compound that enhances hepcidin expression or activity in the system.

86. The method of aspect 84, wherein the candidate compound inhibits the signaling activity of BMP and is identified as a compound that inhibits hepcidin expression or activity in the system.

87. A method for identifying a compound that regulates iron metabolism or an iron metabolic process in a system, wherein the system expresses at least one TGF- β 1 superfamily member, the method comprising steps of: incubating the system with a candidate compound under conditions and for a time sufficient for the candidate compound to modulate the signaling activity of the at least one TGF- β superfamily member, to obtain a test system; measuring, in the test system, at least one factor that is representative of the signaling activity of the at least one TGF- β 1 superfamily member; incubating the system under the same conditions and for the same time absent the candidate compound, to obtain a control system; measuring the factor in the control system; comparing the factor measured in the test and control systems; and determining that the candidate compound regulates iron metabolism or the iron metabolic process, if the factor measured in the test system is less than or greater than the factor measured in the control system.

88. The method of aspect 87, wherein the at least one TGF- β superfamily member is TGF- β or BMP.

89. The method of aspect 88, wherein the system is a cell, a biological fluid, a biological tissue, or an animal.

90. The method of aspect 88, wherein the iron metabolic process is selected from the group consisting of iron uptake, iron absorption, iron transport, iron storage, iron processing, iron mobilization, and iron utilization.

91. The method of aspect 88, wherein the system expresses TGF- β .

92. The method of aspect 91, wherein the candidate compound enhances the signaling activity of TGF- β and is identified as a compound that enhances iron metabolism or the iron metabolic process in the system.

93. The method of aspect 91, wherein the candidate compound inhibits the signaling activity of TGF- β and is identified as a compound that inhibits iron metabolism or the iron metabolic process in the system.

94. The method of aspect 88, wherein the system expresses BMP.

95. The method of aspect 94, wherein the candidate compound enhances the signaling activity of BMP and is identified as a compound that inhibits iron metabolism or the iron metabolic process in the system.

96. The method of aspect 94, wherein the candidate compound inhibits the signaling activity of BMP and is identified as a compound that enhances iron metabolism or the iron metabolic process.

97. A regulator of hepcidin expression or activity identified by the method of aspect 87.

98. A regulator of iron metabolism or an iron metabolic process identified by the method of aspect 87.

99. A pharmaceutical composition comprising an effective amount of at least one regulator of hepcidin expression or activity identified by the method of aspect 87, and a pharmaceutically acceptable carrier.

100. A pharmaceutical composition comprising an effective amount of at least one regulator of iron metabolism or an iron metabolic process identified by the method of aspect 87, and a pharmaceutically acceptable carrier.

101. A method of treating or preventing a condition associated with perturbations in iron metabolism or an iron

metabolic process in a subject, the method comprising administering to the subject an effective amount of at least one regulator of hepcidin expression or activity identified by the method of aspect 87.

5 102. A method of treating or preventing a condition associated with perturbations in iron metabolism or an iron metabolic process in a subject, the method comprising administering to the subject an effective amount of at least one regulator of iron metabolism or an iron metabolic process identified by the method of aspect 87.

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 45 <213> Homo sapiens

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 20 25 30

 55 Ala His Ser Gln Cys Lys Ile Leu Arg Cys Asn Ala Glu Tyr Val Ser
 35 40 45

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	Ser	Thr	Leu	Ser	Leu	Arg	Gly	Gly	Gly	Ser	Ser	Gly	Ala	Leu	Arg	Gly	
	50						55					60					
5	Gly	Gly	Gly	Gly	Gly	Arg	Gly	Gly	Gly	Val	Gly	Ser	Gly	Gly	Leu	Cys	
	65					70					75					80	
	Arg	Ala	Leu	Arg	Ser	Tyr	Ala	Leu	Cys	Thr	Arg	Arg	Thr	Ala	Arg	Thr	
10					85					90					95		
	Cys	Arg	Gly	Asp	Leu	Ala	Phe	His	Ser	Ala	Val	His	Gly	Ile	Glu	Asp	
				100					105					110			
15	Leu	Met	Ile	Gln	His	Asn	Cys	Ser	Arg	Gln	Gly	Pro	Thr	Ala	Pro	Pro	
			115					120					125				
	Pro	Pro	Arg	Gly	Pro	Ala	Leu	Pro	Gly	Ala	Gly	Ser	Gly	Leu	Pro	Ala	
20			130				135						140				
	Pro	Asp	Pro	Cys	Asp	Tyr	Glu	Gly	Arg	Phe	Ser	Arg	Leu	His	Gly	Arg	
25		145				150					155					160	
	Pro	Pro	Gly	Phe	Leu	His	Cys	Ala	Ser	Phe	Gly	Asp	Pro	His	Val	Arg	
				165						170					175		
30	Ser	Phe	His	His	His	Phe	His	Thr	Cys	Arg	Val	Gln	Gly	Ala	Trp	Pro	
				180					185					190			
	Leu	Leu	Asp	Asn	Asp	Phe	Leu	Phe	Val	Gln	Ala	Thr	Ser	Ser	Pro	Met	
35			195					200					205				
	Ala	Leu	Gly	Ala	Asn	Ala	Thr	Ala	Thr	Arg	Lys	Leu	Thr	Ile	Ile	Phe	
40			210				215					220					
	Lys	Asn	Met	Gln	Glu	Cys	Ile	Asp	Gln	Lys	Val	Tyr	Gln	Ala	Glu	Val	
	225					230					235					240	
45	Asp	Asn	Leu	Pro	Val	Ala	Phe	Glu	Asp	Gly	Ser	Ile	Asn	Gly	Gly	Asp	
					245					250					255		
	Arg	Pro	Gly	Gly	Ser	Ser	Leu	Ser	Ile	Gln	Thr	Ala	Asn	Pro	Gly	Asn	
50				260					265					270			
	His	Val	Glu	Ile	Gln	Ala	Ala	Tyr	Ile	Gly	Thr	Thr	Ile	Ile	Ile	Arg	
			275					280					285				
55	Gln	Thr	Ala	Gly	Gln	Leu	Ser	Phe	Ser	Ile	Lys	Val	Ala	Glu	Asp	Val	

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	290		295		300	
5	Ala Met Ala Phe Ser Ala Glu Gln Asp Leu Gln Leu Cys Val Gly Gly					
	305		310		315	320
	Cys Pro Pro Ser Gln Arg Leu Ser Arg Ser Glu Arg Asn Arg Arg Gly					
			325		330	335
10	Ala Ile Thr Ile Asp Thr Ala Arg Arg Leu Cys Lys Glu Gly Leu Pro					
			340		345	350
15	Val Glu Asp Ala Tyr Phe His Ser Cys Val Phe Asp Val Leu Ile Ser					
			355		360	365
	Gly Asp Pro Asn Phe Thr Val Ala Ala Gln Ala Ala Leu Glu Asp Ala					
20			370		375	380
	Arg Ala Phe Leu Pro Asp Leu Glu Lys Leu His Leu Phe Pro Ser Asp					
	385		390		395	400
25	Ala Gly Val Pro Leu Ser Ser Ala Thr Leu Leu Ala Pro Leu Leu Ser					
			405		410	415
	Gly Leu Phe Val Leu Trp Leu Cys Ile Gln					
30			420		425	
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			20		25	30
45	Ala His Ser Gln Cys Lys Ile Leu Arg Cys Asn Ala Glu Tyr Val Ser					
			35		40	45
50	Phe Thr Leu Ser Leu Arg Gly Gly Gly Ser Pro Asp Thr Pro Arg Gly					
	50		55		60	
	Gly Gly Arg Gly Gly Pro Ala Ser Gly Gly Leu Cys Arg Ala Leu Arg					
	65		70		75	80
55	Ser Tyr Ala Leu Cys Thr Arg Arg Thr Ala Arg Thr Cys Arg Gly Asp					

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	85	90	95
5	Leu Ala Phe His Ser Ala Val His Gly Ile Glu Asp Leu Met Ile Gln 100 105 110		
10	His Asn Cys Ser Arg Gln Gly Pro Thr Ala Ser Pro Pro Ala Arg Gly 115 120 125		
15	Pro Ala Leu Pro Gly Ala Gly Pro Ala Pro Leu Thr Pro Asp Pro Cys 130 135 140		
20	Asp Tyr Glu Ala Arg Phe Ser Arg Leu His Gly Arg Thr Pro Gly Phe 145 150 155 160		
25	Leu His Cys Ala Ser Phe Gly Asp Pro His Val Arg Ser Phe His Asn 165 170 175		
30	His Phe His Thr Cys Arg Val Gln Gly Ala Trp Pro Leu Leu Asp Asn 180 185 190		
35	Asp Phe Leu Phe Val Gln Ala Thr Ser Ser Pro Val Ala Ser Gly Ala 195 200 205		
40	Asn Ala Thr Thr Ile Arg Lys Ile Thr Ile Ile Phe Lys Asn Met Gln 210 215 220		
45	Glu Cys Ile Asp Gln Lys Val Tyr Gln Ala Glu Val Asp Asn Leu Pro 225 230 235 240		
50	Ala Ala Phe Glu Asp Gly Ser Val Asn Gly Gly Asp Arg Pro Gly Gly 245 250 255		
55	Ser Ser Leu Ser Ile Gln Thr Ala Asn Leu Gly Ser His Val Glu Ile 260 265 270		
	Arg Ala Ala Tyr Ile Gly Thr Thr Ile Ile Val Arg Gln Thr Ala Gly 275 280 285		
	Gln Leu Ser Phe Ser Ile Arg Val Ala Glu Asp Val Ala Arg Ala Phe 290 295 300		
	Ser Ala Glu Gln Asp Leu Gln Leu Cys Val Gly Gly Cys Pro Pro Ser 305 310 315 320		
	Gln Arg Leu Ser Arg Ser Glu Arg Asn Arg Arg Gly Ala Ile Ala Ile 325 330 335		

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	Asp	Thr	Ala	Arg	Arg	Leu	Cys	Lys	Glu	Gly	Leu	Pro	Val	Glu	Asp	Ala	
				340					345					350			
5	Tyr	Phe	Gln	Ser	Cys	Val	Phe	Asp	Val	Ser	Val	Ser	Gly	Asp	Pro	Asn	
			355					360					365				
10	Phe	Thr	Val	Ala	Ala	Gln	Ser	Ala	Leu	Asp	Asp	Ala	Arg	Val	Phe	Leu	
		370					375					380					
15	Thr	Asp	Leu	Glu	Asn	Leu	His	Leu	Phe	Pro	Val	Asp	Ala	Gly	Pro	Pro	
	385					390					395					400	
20	Leu	Ser	Pro	Ala	Thr	Cys	Leu	Val	Arg	Leu	Leu	Ser	Val	Leu	Phe	Val	
				405						410					415		
25	Leu	Trp	Phe	Cys	Ile	Gln											
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	<211>	420															
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	<400>	26															
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	1				5					10					15		
35	Leu	Ser	Thr	Leu	Thr	Leu	Leu	Leu	Leu	Leu	Cys	Gly	Gln	Ala	His	Ser	
				20					25					30			
40	Gln	Cys	Lys	Ile	Leu	Arg	Cys	Asn	Ala	Glu	Tyr	Val	Ser	Ser	Thr	Leu	
			35					40					45				
45	Ser	Leu	Arg	Gly	Gly	Gly	Ser	Pro	Asp	Thr	Pro	Arg	Gly	Gly	Gly	Arg	
		50					55					60					
50	Gly	Gly	Leu	Ala	Ser	Gly	Gly	Leu	Cys	Arg	Ala	Leu	Arg	Ser	Tyr	Ala	
	65					70				75						80	
55	Leu	Cys	Thr	Arg	Arg	Thr	Ala	Arg	Thr	Cys	Arg	Gly	Asp	Leu	Ala	Phe	
				85						90					95		
60	His	Ser	Ala	Val	His	Gly	Ile	Glu	Asp	Leu	Met	Ile	Gln	His	Asn	Cys	
				100					105					110			
65	Ser	Arg	Gln	Gly	Pro	Thr	Ala	Pro	Pro	Pro	Ala	Arg	Gly	Pro	Ala	Leu	
			115					120					125				

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	Pro Gly Ala Gly Pro Ala Pro Leu Thr Pro Asp Pro Cys Asp Tyr Glu	
	130	135 140
5	Ala Arg Phe Ser Arg Leu His Gly Arg Ala Pro Gly Phe Leu His Cys	
	145	150 155 160
10	Ala Ser Phe Gly Asp Pro His Val Arg Ser Phe His Asn Gln Phe His	
		165 170 175
15	Thr Cys Arg Val Gln Gly Ala Trp Pro Leu Leu Asp Asn Asp Phe Leu	
		180 185 190
20	Phe Val Gln Ala Thr Ser Ser Pro Val Ser Ser Gly Ala Asn Ala Thr	
		195 200 205
25	Thr Ile Arg Lys Ile Thr Ile Ile Phe Lys Asn Met Gln Glu Cys Ile	
		210 215 220
30	Asp Gln Lys Val Tyr Gln Ala Glu Val Asp Asn Leu Pro Ala Ala Phe	
		225 230 235 240
35	Glu Asp Gly Ser Ile Asn Gly Gly Asp Arg Pro Gly Gly Ser Ser Leu	
		245 250 255
40	Ser Ile Gln Thr Ala Asn Leu Gly Ser His Val Glu Ile Arg Ala Ala	
		260 265 270
45	Tyr Ile Gly Thr Thr Ile Ile Ile Arg Gln Thr Ala Gly Gln Leu Ser	
		275 280 285
50	Phe Ser Ile Arg Val Ala Glu Asp Val Ala Arg Ala Phe Ser Ala Glu	
		290 295 300
55	Gln Asp Leu Gln Leu Cys Val Gly Gly Cys Pro Pro Ser Gln Arg Leu	
		305 310 315 320
60	Ser Arg Ser Glu Arg Asn Arg Arg Gly Ala Ile Ala Ile Asp Thr Ala	
		325 330 335
65	Arg Arg Leu Cys Lys Glu Gly Leu Pro Val Glu Asp Ala Tyr Phe Gln	
		340 345 350
70	Ser Cys Val Phe Asp Val Ser Val Ser Gly Asp Pro Asn Phe Thr Val	
		355 360 365
75	Ala Ala Gln Thr Ala Leu Asp Asp Ala Arg Ile Phe Leu Thr Asp Leu	
		370 375 380

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	Glu	Asn	Leu	His	Leu	Phe	Pro	Ser	Asp	Ala	Gly	Pro	Pro	Leu	Ser	Pro	385	390	395	400
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	<211>	408																		
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25	His	Ile	Val	Ile	Ile	Val	Leu	Met	Val	Leu	Leu	Phe	Ser	Ala	Pro	Ser	20	25	30	
30	Val	Cys	Ala	Gln	Cys	Arg	Ile	Leu	Arg	Cys	Asn	Ser	Asp	Phe	Val	Ala	35	40	45	
35	Ala	Thr	Leu	Glu	Ser	Gly	Val	Ile	Gly	Gly	Gly	Asn	Lys	Glu	Gly	Val	50	55	60	
	Asn	Thr	Gly	Tyr	Cys	Ser	Ala	Leu	Arg	Ser	Tyr	Ala	Leu	Cys	Thr	Gln	65	70	75	80
40	Arg	Thr	Ala	Arg	Ala	Cys	Arg	Gly	Asp	Leu	Ala	Tyr	His	Ser	Ala	Val	85	90	95	
45	Gln	Gly	Ile	Glu	Asp	Leu	Leu	Ile	Gln	Tyr	Arg	Cys	Pro	Lys	Ala	Gly	100	105	110	
	Pro	Thr	Ala	Gln	Pro	Gln	Pro	Arg	Pro	Leu	Pro	Gln	Ala	Pro	Leu	Ser	115	120	125	
50	Gly	Asp	Gly	Cys	Phe	Tyr	Glu	Lys	Gly	Phe	Ile	Gln	Arg	Glu	Gly	Arg	130	135	140	
	Ala	Pro	Glu	Tyr	Leu	His	Cys	Gly	Val	Phe	Gly	Asp	Pro	His	Ile	Arg	145	150	155	160
55	Thr	Phe	Asn	Glu	Glu	Phe	Gln	Thr	Cys	Ala	Val	Gln	Gly	Ala	Trp	Pro	165	170	175	

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	Leu	Ile	Asp	Asn	Gln	Tyr	Leu	Tyr	Ile	Gln	Ala	Thr	Ser	Ser	Pro	Thr	
				180					185						190		
5	Arg	Glu	Ser	Ser	Asp	Thr	Thr	Ile	Leu	Thr	Glu	Val	Thr	Val	Ile	Phe	
			195					200					205				
10	Gln	Asn	Trp	Arg	Glu	Cys	Ala	Glu	Gln	Gln	Val	Tyr	Gln	Ala	Lys	Leu	
		210					215					220					
15	Gly	Asn	Val	Pro	Pro	Ala	Phe	Ala	Asp	Gly	Ser	Val	Thr	Gly	Gly	Asp	
	225					230					235					240	
20	Arg	Arg	Gly	His	Gln	Ser	Leu	Arg	Ile	His	Ser	Gln	Asp	Pro	Gly	Arg	
				245						250					255		
25	His	Ala	Glu	Ile	Trp	Ala	Thr	His	Ile	Gly	Thr	Met	Ile	Ile	Val	Arg	
			260						265					270			
30	Gln	Val	Gly	Gln	Ser	Leu	Ser	Leu	Ser	Val	Arg	Ser	Pro	Arg	Ala	Ile	
		275					280						285				
35	Val	Glu	Ser	Tyr	Thr	Pro	Glu	Gln	Asp	Leu	Gln	Leu	Cys	Val	Trp	Gly	
	290						295					300					
40	Cys	Pro	Ile	Ser	Gln	Arg	Leu	Glu	Met	Leu	His	Ala	His	Pro	Phe	Asp	
	305					310					315					320	
45	Pro	Ala	Tyr	Thr	His	Cys	Ser	Ser	Leu	Phe	Pro	Gly	Arg	Asp	Val	Tyr	
				325						330					335		
50	Phe	Gln	Ala	Cys	Leu	Phe	Asp	Val	Gln	Val	Thr	Gly	Asp	Val	Asn	Ser	
			340						345					350			
55	Ser	Ala	Ser	Ala	Val	Ala	Ala	Leu	Glu	Asp	Ala	Arg	Ala	Met	Ile	Ser	
		355						360					365				
60	Asp	Pro	Ala	Ser	Val	His	Leu	Val	Thr	Gly	Gly	Thr	Gly	Asn	Asn	Ser	
	370					375						380					
65	Pro	Ser	Leu	Leu	Val	Val	Leu	Gly	Phe	Ser	Phe	Leu	Thr	Glu	Thr	Leu	
	385				390						395					400	
70	Arg	His	Ser	Phe	Leu	Gly	Ser	Ala									
				405													
75	<210>	28															
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<212> PRT
 <213> Homo sapiens
 <400> 28

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15	Leu	Lys	Cys	Asn	Ser	Glu	Phe	Trp	Ser	Ala	Thr	Ser	Gly	Ser	His	Ala	35	40	45	
20	Pro	Ala	Ser	Asp	Asp	Thr	Pro	Glu	Phe	Cys	Ala	Ala	Leu	Arg	Ser	Tyr	50	55	60	
25	Ala	Leu	Cys	Thr	Arg	Arg	Thr	Ala	Arg	Thr	Cys	Arg	Gly	Asp	Leu	Ala	65	70	75	80
30	Tyr	His	Ser	Ala	Val	His	Gly	Ile	Glu	Asp	Leu	Met	Ser	Gln	His	Asn	85	90	95	
35	Cys	Ser	Lys	Asp	Gly	Pro	Thr	Ser	Gln	Pro	Arg	Leu	Arg	Thr	Leu	Pro	100	105	110	
40	Pro	Ala	Gly	Asp	Ser	Gln	Glu	Arg	Ser	Asp	Ser	Pro	Glu	Ile	Cys	His	115	120	125	
45	Tyr	Glu	Lys	Ser	Phe	His	Lys	His	Ser	Ala	Thr	Pro	Asn	Tyr	Thr	His	130	135	140	
50	Cys	Gly	Leu	Phe	Gly	Asp	Pro	His	Leu	Arg	Thr	Phe	Thr	Asp	Arg	Phe	145	150	155	160
55	Gln	Thr	Cys	Lys	Val	Gln	Gly	Ala	Trp	Pro	Leu	Ile	Asp	Asn	Asn	Tyr	165	170	175	
	Leu	Asn	Val	Gln	Ala	Thr	Asn	Thr	Pro	Val	Leu	Pro	Gly	Ser	Ala	Ala	180	185	190	
	Thr	Ala	Thr	Ser	Lys	Leu	Thr	Ile	Ile	Phe	Lys	Asn	Phe	Gln	Glu	Cys	195	200	205	
	Val	Asp	Gln	Lys	Val	Tyr	Gln	Ala	Glu	Met	Asp	Glu	Leu	Pro	Ala	Ala	210	215	220	
	Phe	Val	Asp	Gly	Ser	Lys	Asn	Gly	Gly	Asp	Lys	His	Gly	Ala	Asn	Ser	225	230	235	240

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	Leu	Lys	Ile	Thr	Glu	Lys	Val	Ser	Gly	Gln	His	Val	Glu	Ile	Gln	Ala	
					245					250					255		
5	Lys	Tyr	Ile	Gly	Thr	Thr	Ile	Val	Val	Arg	Gln	Val	Gly	Arg	Tyr	Leu	
				260					265					270			
10	Thr	Phe	Ala	Val	Arg	Met	Pro	Glu	Glu	Val	Val	Asn	Ala	Val	Glu	Asp	
			275					280					285				
15	Trp	Asp	Ser	Gln	Gly	Leu	Tyr	Leu	Cys	Leu	Arg	Gly	Cys	Pro	Leu	Asn	
		290					295					300					
20	Gln	Gln	Ile	Asp	Phe	Gln	Ala	Phe	His	Thr	Asn	Ala	Glu	Gly	Thr	Gly	
	305					310					315					320	
25	Ala	Arg	Arg	Leu	Ala	Ala	Ala	Ser	Pro	Ala	Pro	Thr	Ala	Pro	Glu	Thr	
					325					330					335		
30	Phe	Pro	Tyr	Glu	Thr	Ala	Val	Ala	Lys	Cys	Lys	Glu	Lys	Leu	Pro	Val	
				340					345					350			
35	Glu	Asp	Leu	Tyr	Tyr	Gln	Ala	Cys	Val	Phe	Asp	Leu	Leu	Thr	Thr	Gly	
			355					360					365				
40	Asp	Val	Asn	Phe	Thr	Leu	Ala	Ala	Tyr	Tyr	Ala	Leu	Glu	Asp	Val	Lys	
		370					375					380					
45	Met	Leu	His	Ser	Asn	Lys	Asp	Lys	Leu	His	Leu	Tyr	Glu	Arg	Thr	Arg	
	385					390					395					400	
50	Asp	Leu	Pro	Gly	Arg	Ala	Ala	Ala	Gly	Leu	Pro	Leu	Ala	Pro	Arg	Pro	
					405					410					415		
55	Leu	Leu	Gly	Ala	Leu	Val	Pro	Leu	Leu	Ala	Leu	Leu	Pro	Val	Phe	Cys	
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75	<213>	Gallus gallus															
80	<400>	29															
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	1				5					10					15		
90	Pro	Val	Phe	Leu	Cys	Ile	Phe	Pro	Pro	Val	Thr	Ser	Pro	Cys	Lys	Ile	
				20					25					30			

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	Leu	Lys	Cys	Asn	Ser	Glu	Phe	Trp	Ala	Ala	Thr	Ser	Gly	Ser	His	His	
			35					40					45				
5	Leu	Gly	Ala	Glu	Glu	Thr	Pro	Glu	Phe	Cys	Thr	Ala	Leu	Arg	Ala	Tyr	
		50					55					60					
	Ala	His	Cys	Thr	Arg	Arg	Thr	Ala	Arg	Thr	Cys	Arg	Gly	Asp	Leu	Ala	
10	65					70					75					80	
	Tyr	His	Ser	Ala	Val	His	Gly	Ile	Asp	Asp	Leu	Met	Val	Gln	His	Asn	
					85					90					95		
15	Cys	Ser	Lys	Asp	Gly	Pro	Thr	Ser	Gln	Pro	Arg	Leu	Arg	Thr	Leu	Pro	
				100					105					110			
	Pro	Gly	Asp	Ser	Gln	Glu	Arg	Ser	Asp	Ser	Pro	Glu	Ile	Cys	His	Tyr	
20			115						120				125				
	Glu	Lys	Ser	Phe	His	Lys	His	Ser	Ala	Ala	Pro	Asn	Tyr	Thr	His	Cys	
25		130					135					140					
	Gly	Leu	Phe	Gly	Asp	Pro	His	Leu	Arg	Thr	Phe	Thr	Asp	Thr	Phe	Gln	
	145					150					155					160	
30	Thr	Cys	Lys	Val	Gln	Gly	Ala	Trp	Pro	Leu	Ile	Asp	Asn	Asn	Tyr	Leu	
					165					170					175		
	Asn	Val	Gln	Val	Thr	Asn	Thr	Pro	Val	Leu	Pro	Gly	Ser	Ser	Ala	Thr	
35				180					185					190			
	Ala	Thr	Ser	Lys	Leu	Thr	Ile	Ile	Phe	Lys	Ser	Phe	Gln	Glu	Cys	Val	
			195					200					205				
40	Glu	Gln	Lys	Val	Tyr	Gln	Ala	Glu	Met	Asp	Glu	Leu	Pro	Ala	Ala	Phe	
		210					215					220					
	Ala	Asp	Gly	Ser	Lys	Asn	Gly	Gly	Asp	Lys	His	Gly	Ala	Asn	Ser	Leu	
45	225					230					235					240	
	Lys	Ile	Thr	Glu	Lys	Val	Ser	Gly	Gln	His	Ile	Glu	Ile	Gln	Ala	Lys	
50					245					250					255		
	Tyr	Ile	Gly	Thr	Thr	Ile	Val	Val	Arg	Gln	Val	Gly	Arg	Tyr	Leu	Thr	
				260					265					270			
55	Phe	Ala	Val	Arg	Met	Pro	Glu	Glu	Val	Val	Asn	Ala	Val	Glu	Asp	Arg	

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	275		280		285
5	Asp Ser Gln Gly Leu Tyr Leu Cys Leu Arg Gly Cys Pro Leu Asn Gln				
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10	Gln Ile Asp Phe Gln Thr Phe Arg Leu Ala Gln Ala Ala Glu Gly Arg				
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15	Ala Arg Arg Lys Gly Pro Ser Leu Pro Ala Pro Pro Glu Ala Phe Thr				
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20	Tyr Glu Ser Ala Thr Ala Lys Cys Arg Glu Lys Leu Pro Val Glu Asp				
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25	Leu Tyr Phe Gln Ser Cys Val Phe Asp Leu Leu Thr Thr Gly Asp Val				
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30	Asn Phe Met Leu Ala Ala Tyr Tyr Ala Phe Glu Asp Val Lys Met Leu				
		370		375	380
35	His Ser Asn Lys Asp Lys Leu His Leu Tyr Glu Arg Thr Arg Ala Leu				
		385		390	395
40	Ala Pro Gly Asn Ala Ala Pro Ser Glu His Pro Trp Ala Leu Pro Ala				
		405		410	415
45	Leu Trp Val Ala Leu Leu Ser Leu Ser Gln Cys Trp Leu Gly Leu Leu				
		420		425	430
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55	<400> 30				
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60	Val Glu Gln Arg Arg Ser Pro Gly Leu Cys Pro Pro Pro Leu Glu Leu				
		20		25	30
65	Leu Leu Leu Leu Leu Phe Ser Leu Gly Leu Leu His Ala Gly Asp Cys				
		35		40	45
70	Gln Gln Pro Ala Gln Cys Arg Ile Gln Lys Cys Thr Thr Asp Phe Val				
		50		55	60
75	Ser Leu Thr Ser His Leu Asn Ser Ala Val Asp Gly Phe Asp Ser Glu				

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	65					70						75				80
5	Phe	Cys	Lys	Ala	Leu	Arg	Ala	Tyr	Ala	Gly	Cys	Thr	Gln	Arg	Thr	Ser
					85					90					95	
10	Lys	Ala	Cys	Arg	Gly	Asn	Leu	Val	Tyr	His	Ser	Ala	Val	Leu	Gly	Ile
				100					105					110		
15	Ser	Asp	Leu	Met	Ser	Gln	Arg	Asn	Cys	Ser	Lys	Asp	Gly	Pro	Thr	Ser
			115					120					125			
20	Ser	Thr	Asn	Pro	Glu	Val	Thr	His	Asp	Pro	Cys	Asn	Tyr	His	Ser	His
		130					135					140				
25	Ala	Gly	Ala	Arg	Glu	His	Arg	Arg	Gly	Asp	Gln	Asn	Pro	Pro	Ser	Tyr
	145					150					155					160
30	Leu	Phe	Cys	Gly	Leu	Phe	Gly	Asp	Pro	His	Leu	Arg	Thr	Phe	Lys	Asp
					165					170					175	
35	Asn	Phe	Gln	Thr	Cys	Lys	Val	Glu	Gly	Ala	Trp	Pro	Leu	Ile	Asp	Asn
				180					185					190		
40	Asn	Tyr	Leu	Ser	Val	Gln	Val	Thr	Asn	Val	Pro	Val	Val	Pro	Gly	Ser
			195					200					205			
45	Ser	Ala	Thr	Ala	Thr	Asn	Lys	Ile	Thr	Ile	Ile	Phe	Lys	Ala	His	His
		210					215					220				
50	Glu	Cys	Thr	Asp	Gln	Lys	Val	Tyr	Gln	Ala	Val	Thr	Asp	Asp	Leu	Pro
	225					230					235				240	
55	Ala	Ala	Phe	Val	Asp	Gly	Thr	Thr	Ser	Gly	Gly	Asp	Ser	Asp	Ala	Lys
					245					250					255	
60	Ser	Leu	Arg	Ile	Val	Glu	Arg	Glu	Ser	Gly	His	Tyr	Val	Glu	Met	His
				260					265					270		
65	Ala	Arg	Tyr	Ile	Gly	Thr	Thr	Val	Phe	Val	Arg	Gln	Val	Gly	Arg	Tyr
			275					280					285			
70	Leu	Thr	Leu	Ala	Ile	Arg	Met	Pro	Glu	Asp	Leu	Ala	Met	Ser	Tyr	Glu
		290					295					300				
75	Glu	Ser	Gln	Asp	Leu	Gln	Leu	Cys	Val	Asn	Gly	Cys	Pro	Leu	Ser	Glu
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Leu Pro Arg Thr Ser Leu Val Gln Ala Trp Pro Gly Tyr Thr Leu Glu
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Thr Ala Asn Thr Gln Cys His Glu Lys Met Pro Val Lys Asp Ile Tyr
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Thr Ala Ala Ala His Ser Ala Leu Glu Asp Val Glu Ala Leu His Pro
385 390 395 400

Arg Lys Glu Arg Trp His Ile Phe Pro Ser Ser Gly Asn Gly Thr Pro
405 410 415

Arg Gly Gly Ser Asp Leu Ser Val Ser Leu Gly Leu Thr Cys Leu Ile
420 425 430

Leu Ile Val Phe Leu
435

Claims

1. A BMP antagonist for use in a method of inhibiting hepcidin expression or activity in a mammalian subject exhibiting iron deficiency, wherein said BMP antagonist is for administration in an amount effective to inhibit hepcidin expression or activity in said mammalian subject.
2. The BMP antagonist for use according to claim 1, wherein the BMP antagonist is an antibody or fragment thereof.
3. The BMP antagonist for use according to claim 1 or claim 2, wherein the BMP antagonist is a Fab fragment, F(ab')₂ fragment or single chain variable fragment (scFv).
4. The BMP antagonist for use according to any preceding claim, wherein the BMP antagonist antagonizes BMP-2 or BMP-4.
5. The BMP antagonist for use according to any preceding claim, wherein the BMP antagonist is for use in a method of inhibiting hepcidin expression or activity for the prevention or treatment of a condition associated with iron deficiency in the subject.
6. The BMP antagonist for use according to any preceding claim, wherein the iron deficiency is selected from the group consisting of anemia of chronic disease, iron deficiency anemia, functional iron deficiency, and microcytic anemia.
7. The BMP antagonist for use according to claim 5, wherein the condition associated with iron deficiency is anemia of chronic disease, and wherein said anemia of chronic disease is associated with chronic bacterial endocarditis, osteomyelitis, rheumatic fever, ulcerative colitis, a neoplastic disorder, infection, or inflammation.
8. The BMP antagonist for use according to claim 6, wherein the anemia of chronic disease is associated with infection, and wherein said infection is a pulmonary abscess, or tuberculosis.

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9. The BMP antagonist for use according to claim 6, wherein the anemia of chronic disease is associated with inflammation, and wherein said inflammation is caused by rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, hepatitis, or inflammatory bowel disease.

5 10. The BMP antagonist for use according to any preceding claim, wherein the mammalian subject is a human.

11. The BMP antagonist for use according to any preceding claim, wherein the subject exhibits low hematocrit associated with iron deficiency.

10 12. The BMP antagonist for use according to any preceding claim, wherein the subject exhibits low serum hemoglobin associated with iron deficiency.

13. The BMP antagonist for use according to any preceding claim, wherein the antibody antagonizes BMP-2.

15 14. The BMP antagonist for use according to any preceding claim, wherein the antibody antagonizes BMP-4.

15. A composition comprising the BMP antagonist for use according to any of claims 1 to 14.

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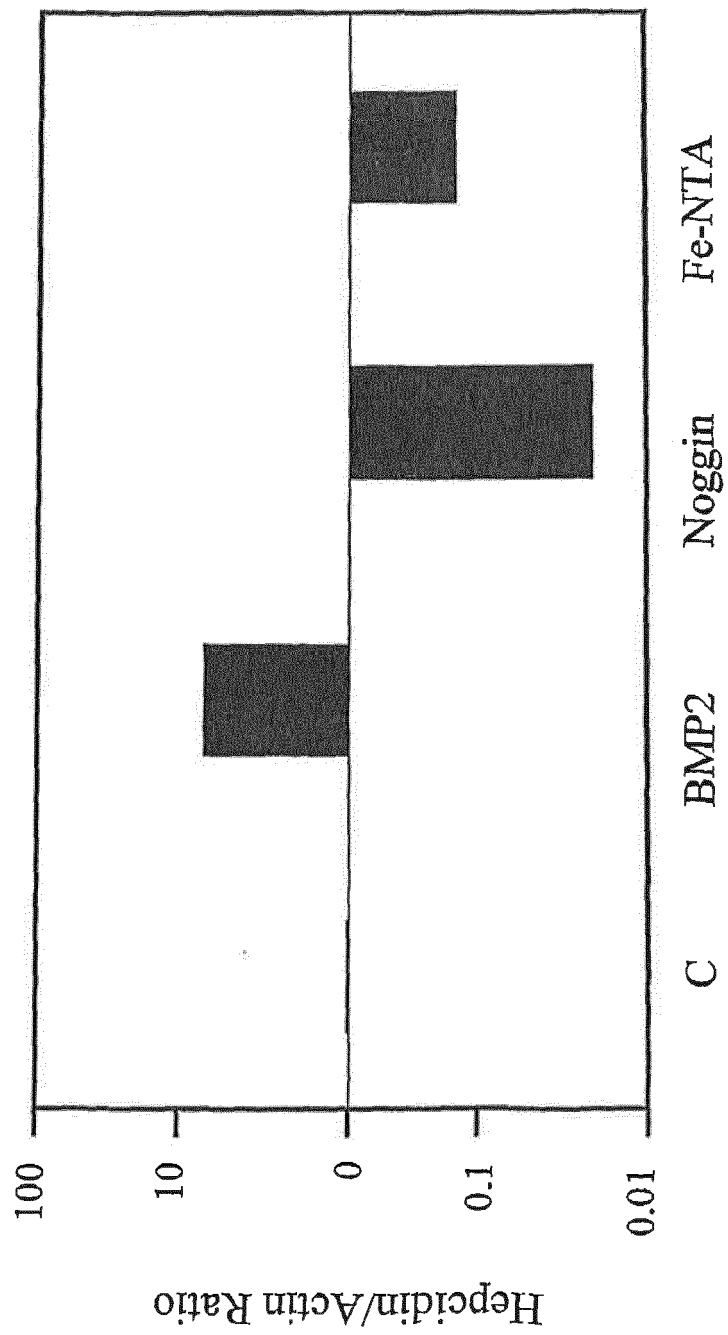


Figure 1

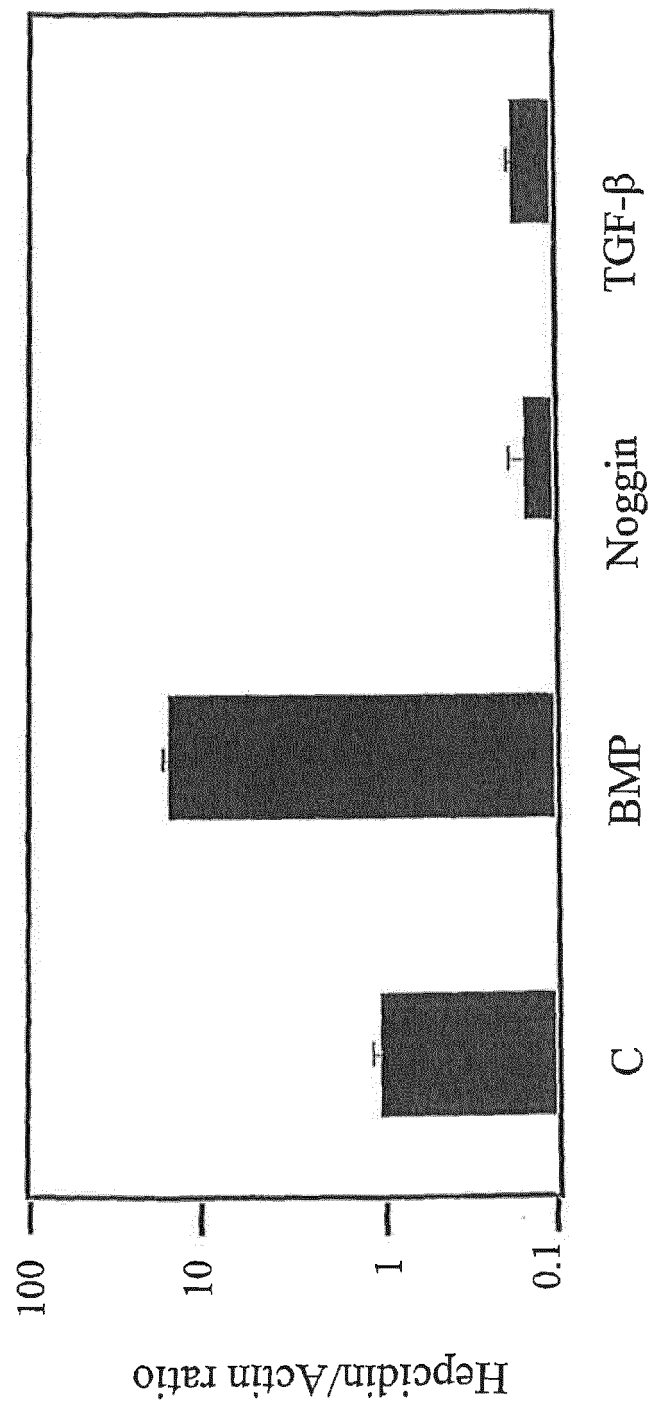


Figure 2

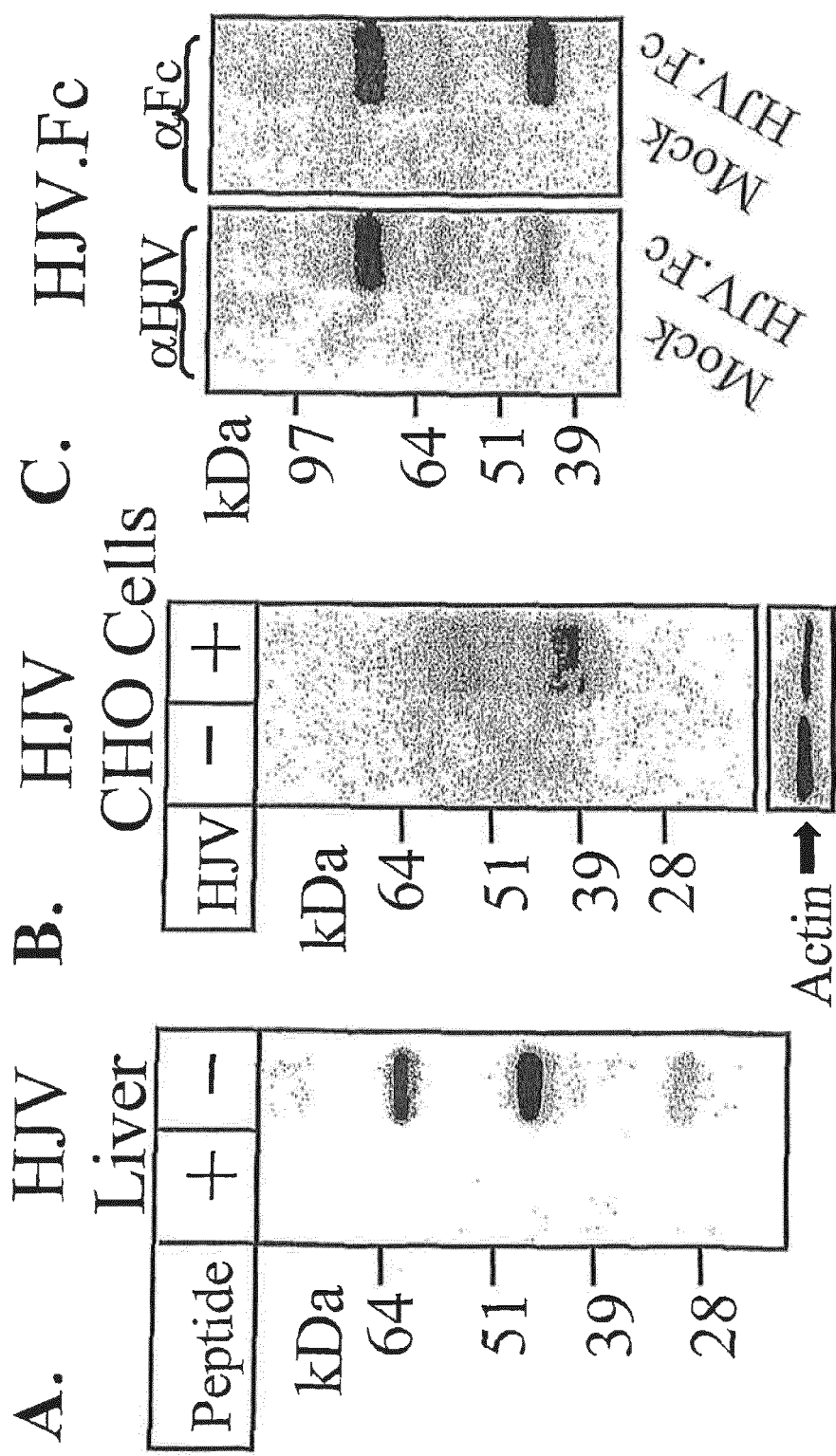


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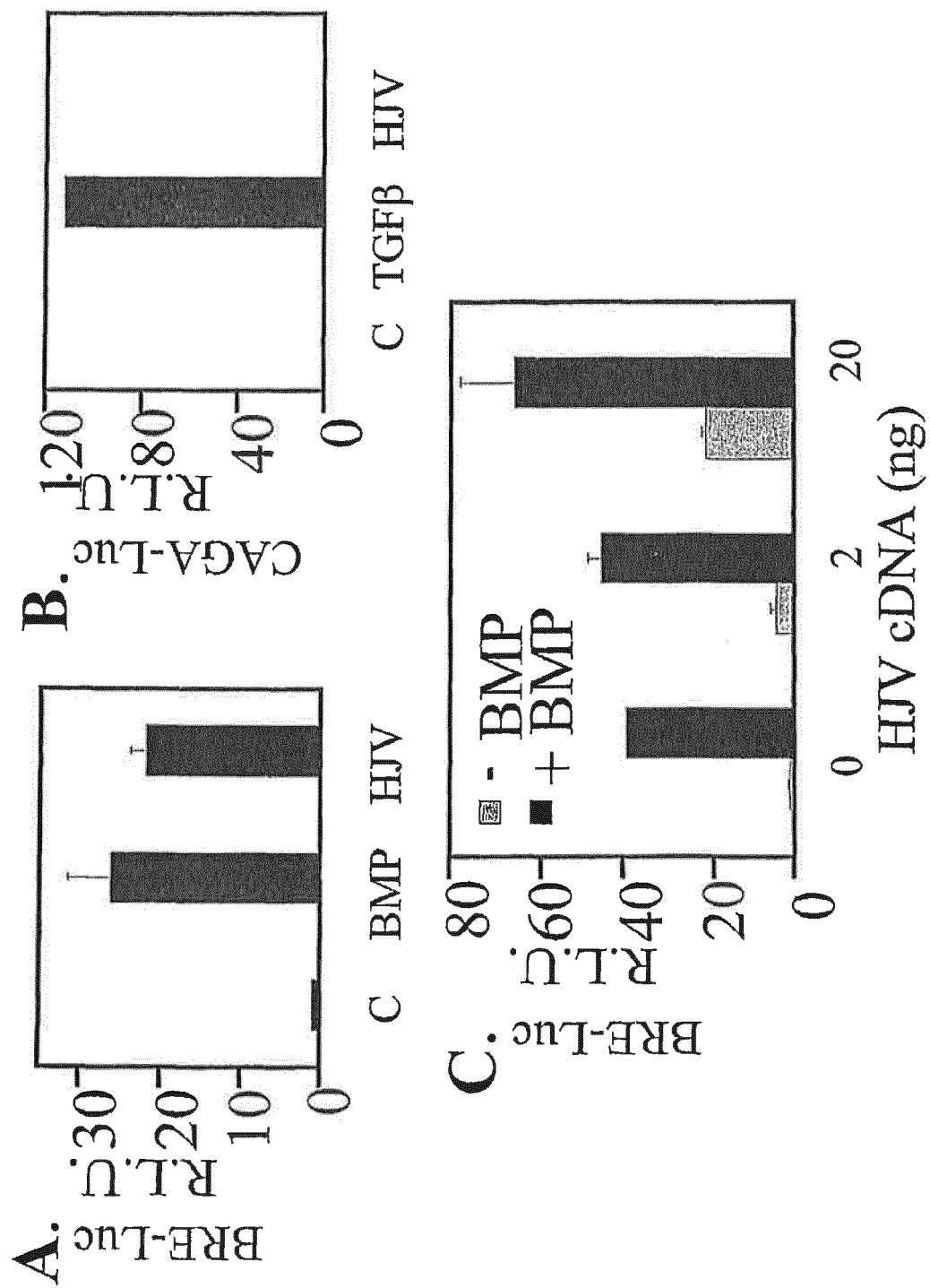


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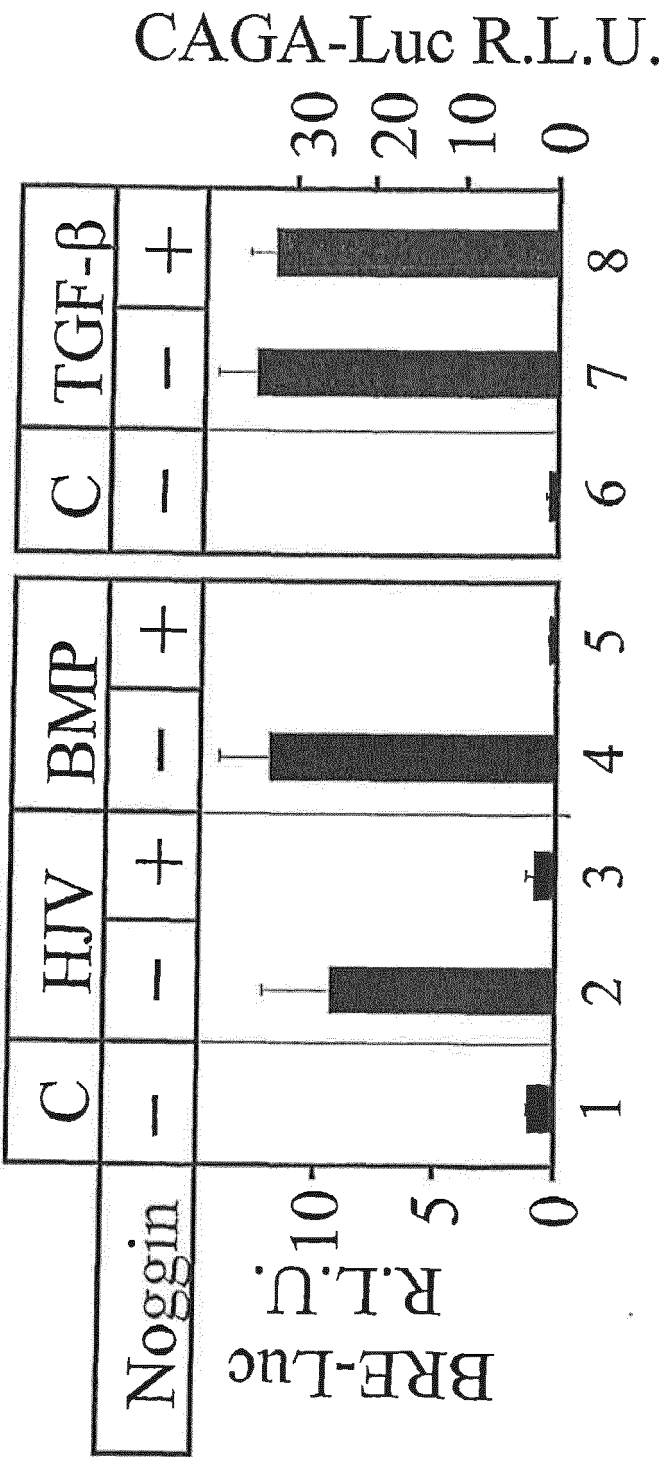


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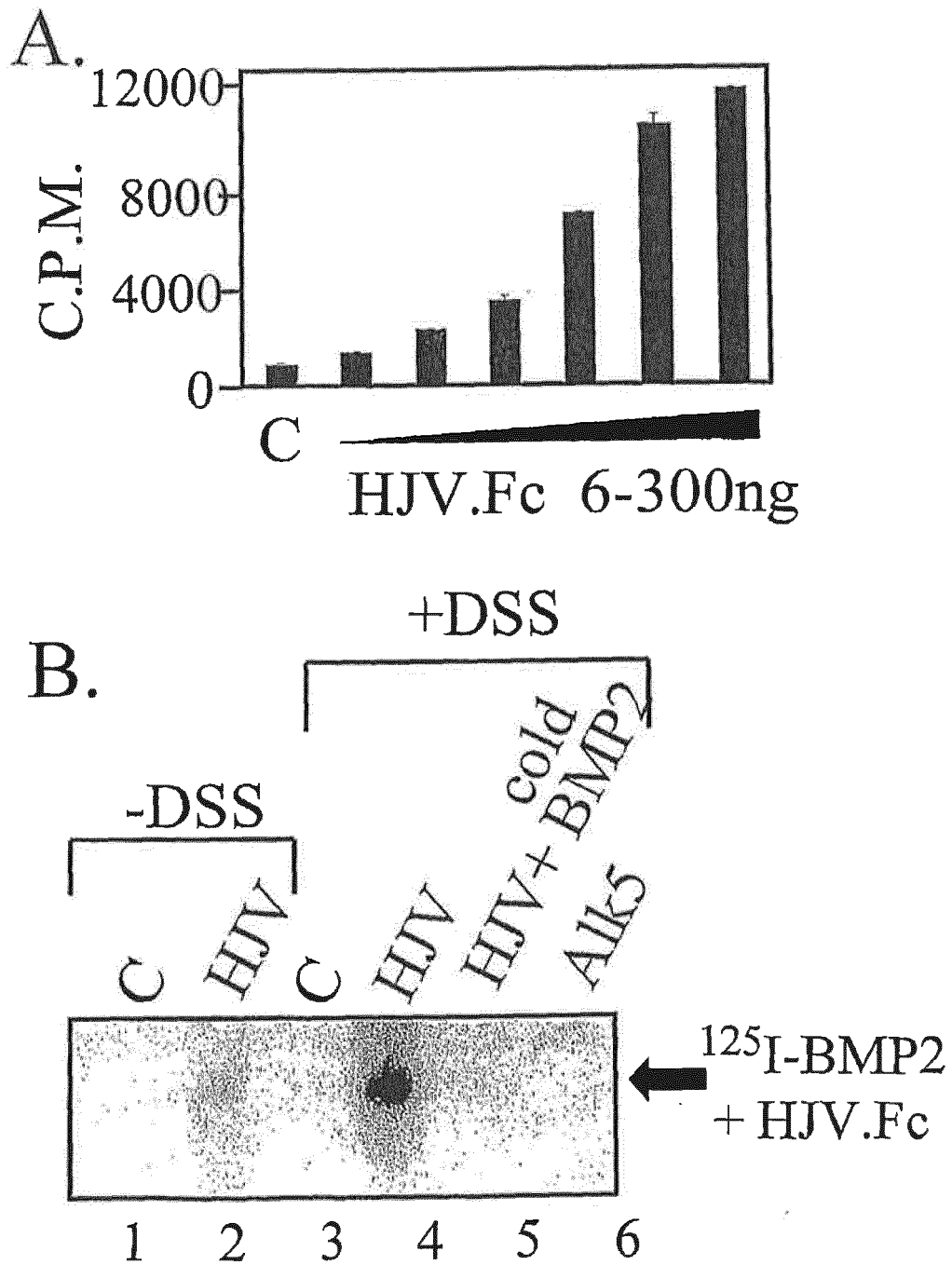


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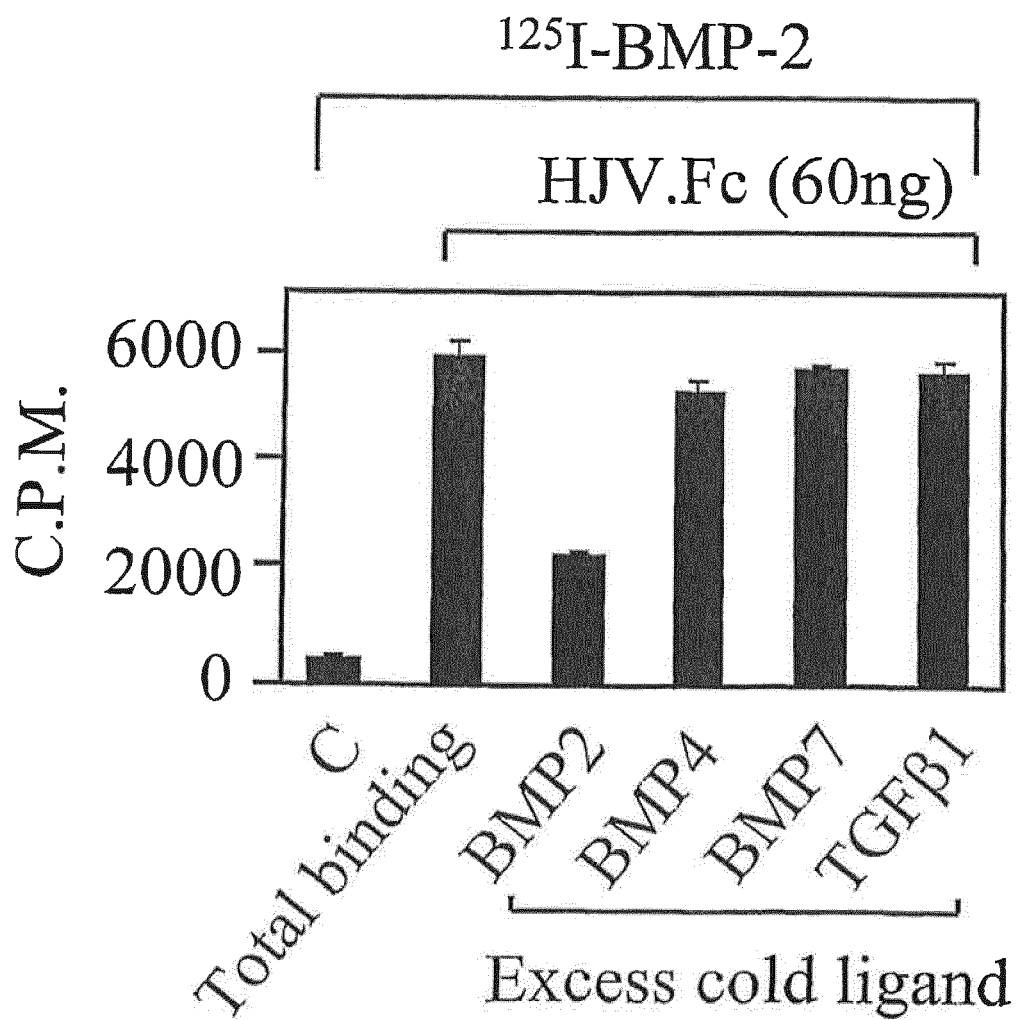


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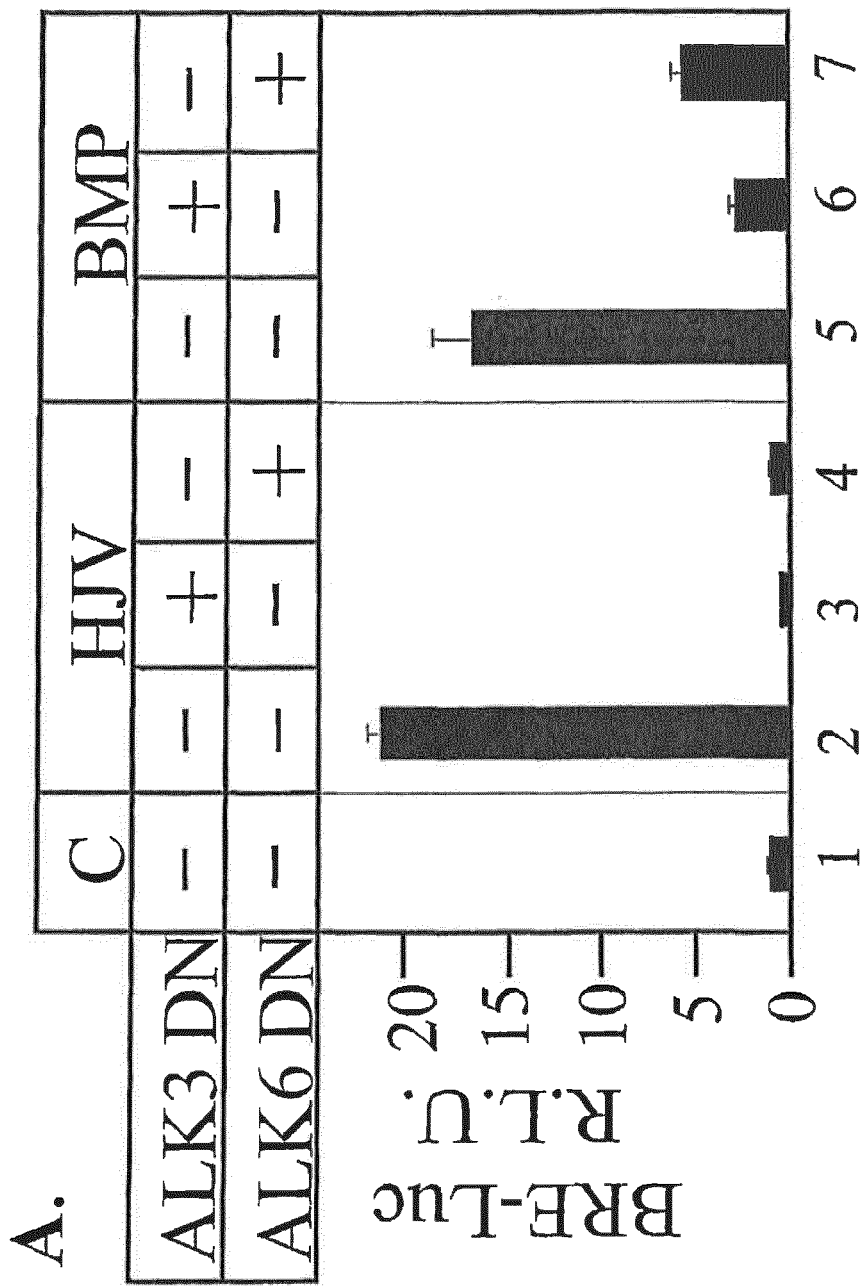


Figure 8(A)

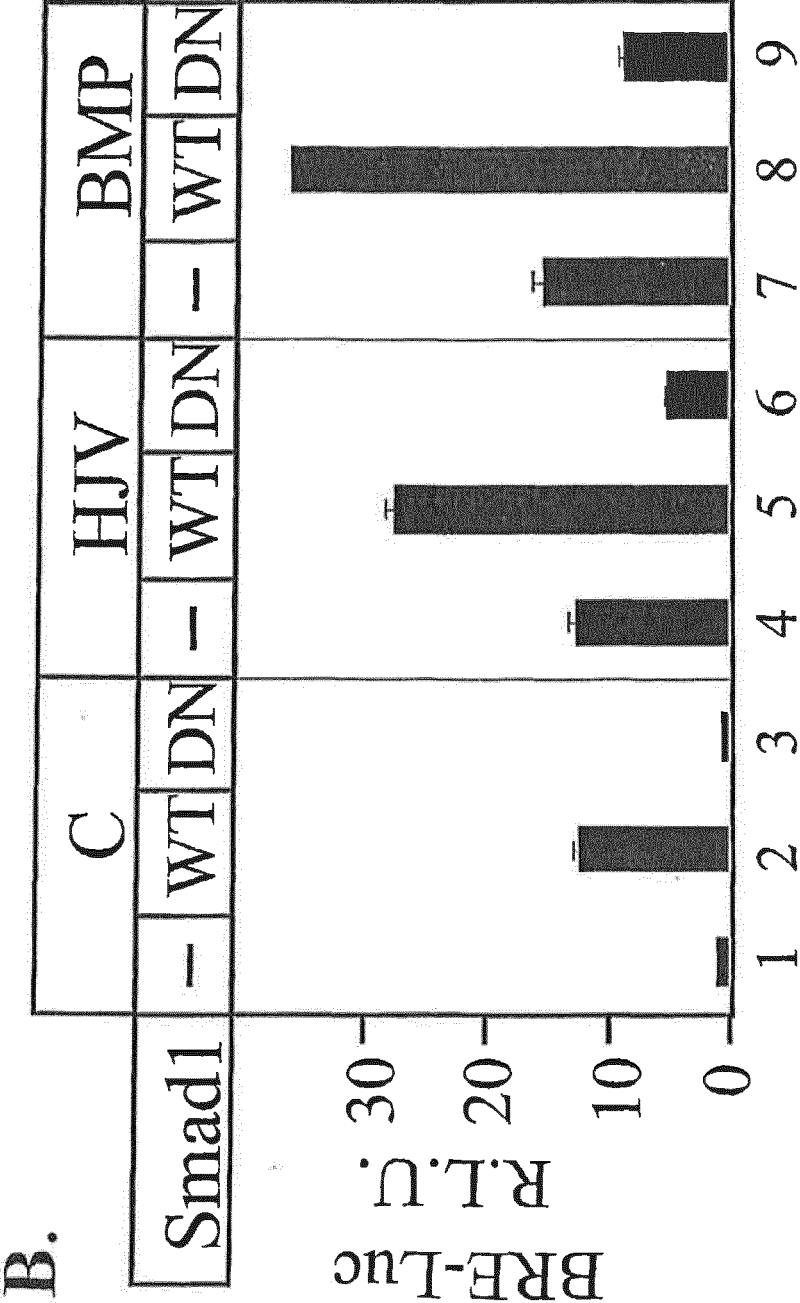


Figure 8(B)

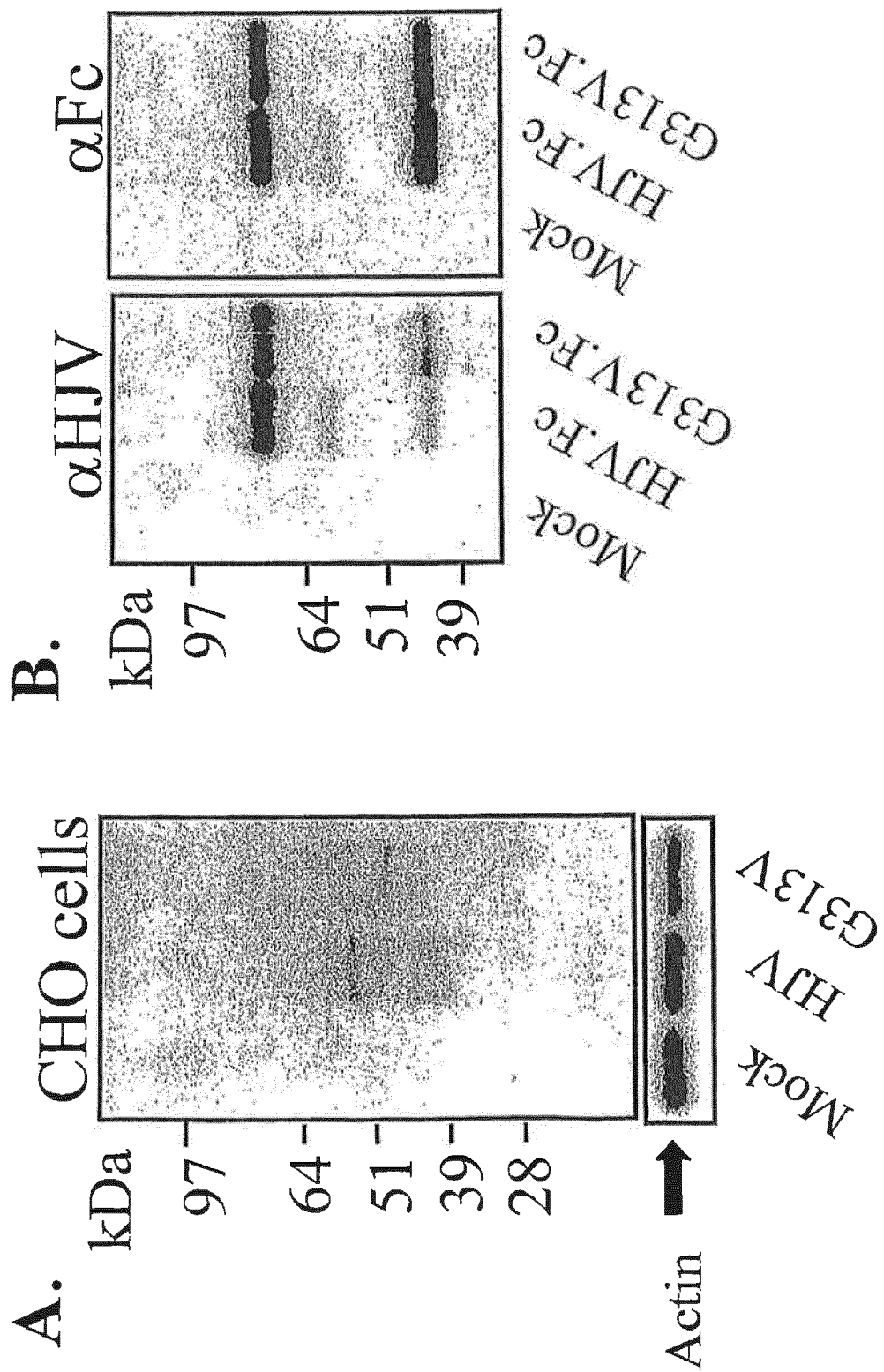


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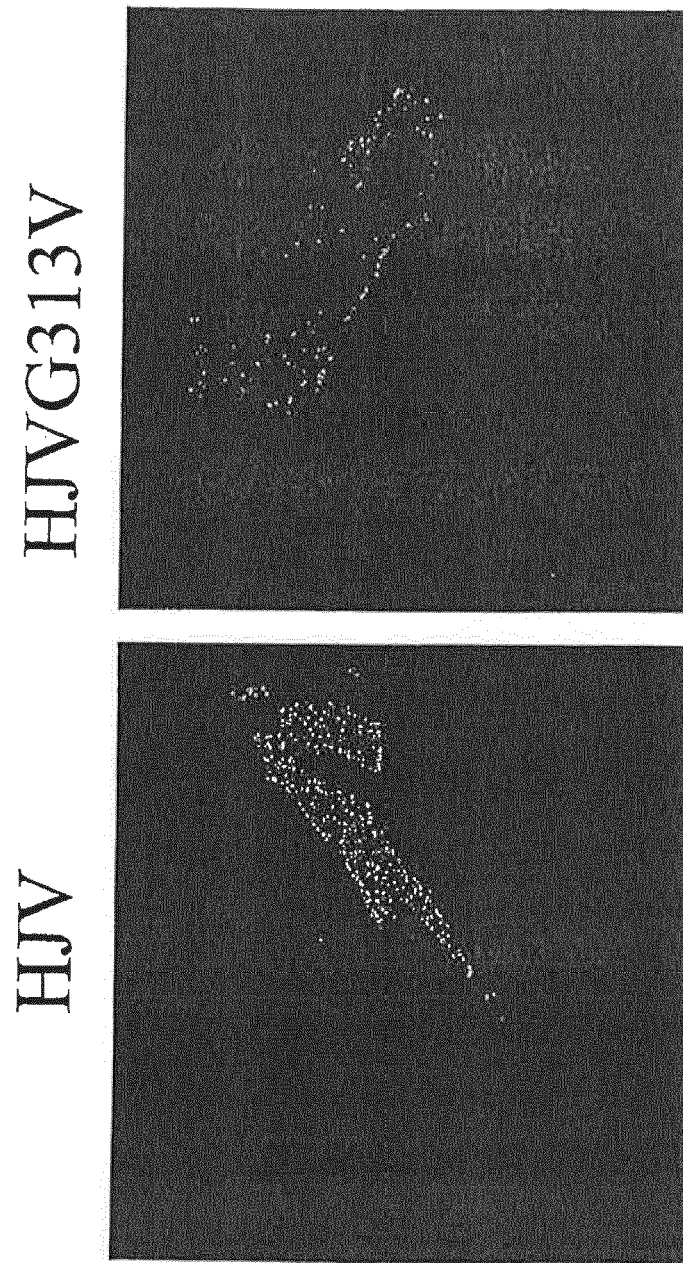


Figure 10

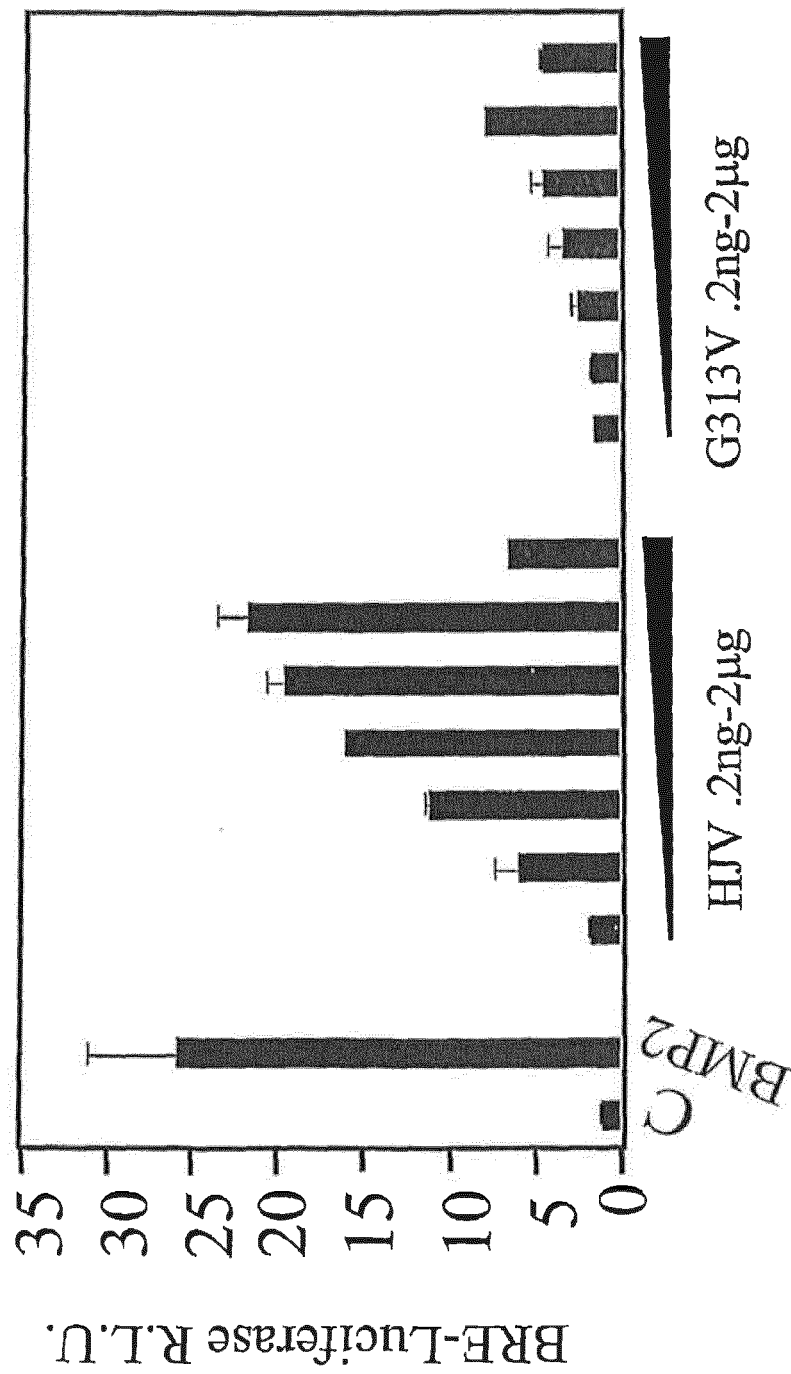


Figure 11

	Serum Iron	TIBC	
BMP2 (18 mcg)	114.02	216.80	n=8 mice
Vehicle	169.12	249.09	n=7 mice
p value	0.0194	0.0268	

Figure 12

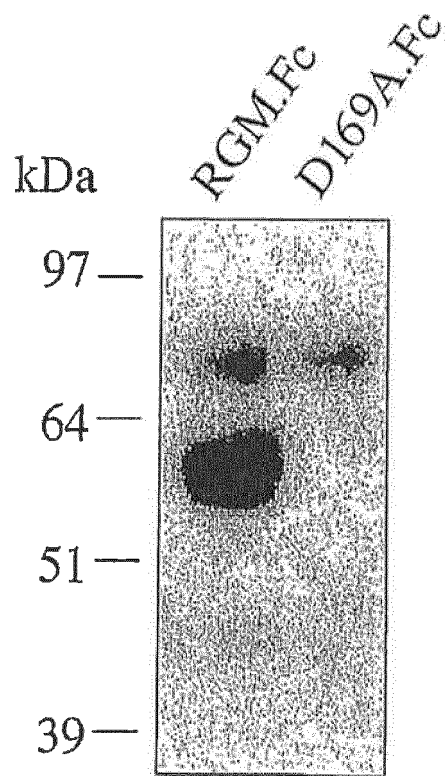


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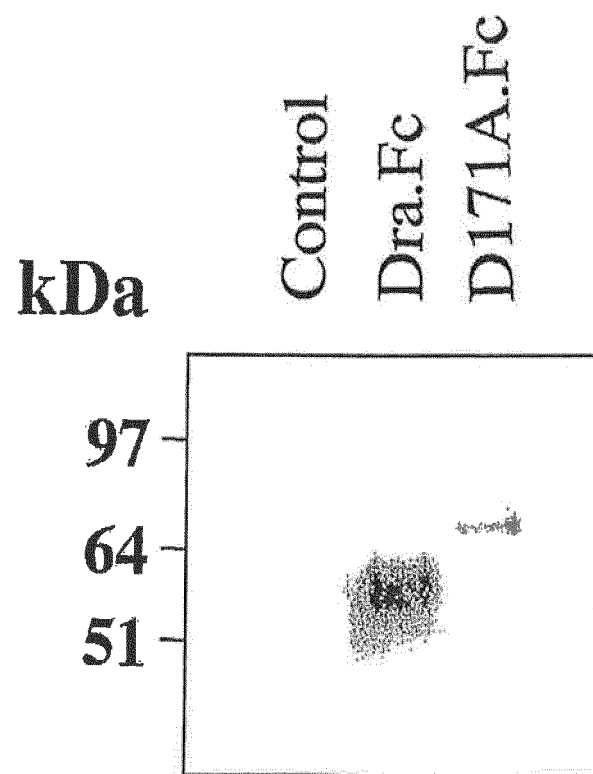


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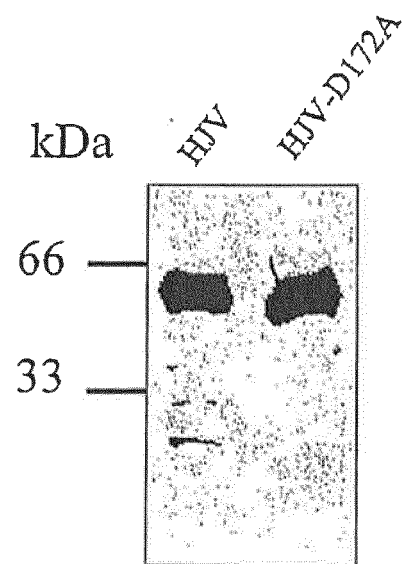


Figure 15



EUROPEAN SEARCH REPORT

Application Number
EP 15 17 3396

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X,P	LIN LAN ET AL: "Competitive regulation of hepcidin mRNA by soluble and cell-associated hemojuvelin", BLOOD, vol. 106, no. 8, 5 July 2005 (2005-07-05), pages 2884-2889, XP002745240, ISSN: 0006-4971	15	INV. A61K38/17 A61K47/48 A61K39/395 A61P3/02 A61P7/06 C07K14/705 C07K16/28
Y,P	* see abstract and page 2889 *	1-15	
X	SAMAD TAREK A ET AL: "DRAGON, a bone morphogenetic protein co-receptor", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 280, no. 14, 25 January 2005 (2005-01-25), pages 14122-14129, XP002476345, ISSN: 0021-9258, DOI: 10.1074/JBC.M410034200 [retrieved on 2005-01-25]	15	
Y	* the whole document *	1-15	TECHNICAL FIELDS SEARCHED (IPC)
T	BILLY ANDRIOPOULOS JR ET AL: "BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism", NATURE GENETICS, NATURE PUBLISHING GROUP, NEW YORK, US, vol. 41, no. 4, 1 April 2009 (2009-04-01), pages 482-487, XP008147148, ISSN: 1061-4036, DOI: 10.1038/NG.335 [retrieved on 2009-03-01] * abstract *		A61K A61P C07K
The present search report has been drawn up for all claims			
Place of search Munich		Date of completion of the search 30 September 2015	Examiner Merckling-Ruiz, V
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

EPO FORM 1503 03.02 (P04C01)

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摘要

本發明提供用於調節哺乳動物中鐵代謝的新的系統和策略。特別地，描述了使用TGF- β 超家族成員的激動劑和拮抗劑調節鐵調素的表達或活性的方法，鐵調素為一種鐵代謝的主要調節劑。本發明的方法在治療與鐵超負荷相關的疾病中和治療與缺鐵症相關的疾病中得到了應用，所述與鐵超負荷相關的疾病比如青少年血色病和成年人血色病，所述缺鐵症比如慢性病貧血和腎病末期的EPO抗性貧血。本發明還涉及篩選研究用於治療鐵代謝病症的新的藥物和治療劑的工具和方法。