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(54) Title: METHODS AND COMPOSITIONS FOR TARGETING A CELL

(57) Abstract: Oligomeric ligands, such as cysteine knot growth factors, can be displayed on the surface of viral particles as recombinant fusion proteins. The recombinant fusion proteins can be used to screen for drugs which will inhibit binding of an oligomeric ligand to its receptor and for targeting transferable labels, including therapeutic gene products, to cells bearing a receptor specific for the oligomeric ligand.

Title: Methods and Compositions for Targeting a Cell

Field of the Invention

The invention relates to the display of oligomeric ligands, particularly growth factors, as recombinant fusion proteins on viral surfaces.

Background of the Invention

Retroviruses and retroviral vectors can efficiently transfer genes into eukaryotic cells. Gene delivery is initiated by retroviral envelope glycoproteins (Env), which mediate attachment to cell-surface receptors and subsequent fusion between viral and cellular membranes. Env proteins are homooligomers containing two to four heterodimeric subunits (Doms *et al.*, 1993). In the case of murine leukemia viruses (MLV), the envelope proteins are homotrimers (Kamps *et al.*, 1991). Each Env subunit comprises a large glycoprotein (SU), which mediates attachment to a cellular receptor, and a smaller transmembrane component (TM), which is responsible for fusion between viral and cellular membranes. The C-terminal end of SU is non-covalently linked to TM. 4070A (amphotropic) murine leukemia virus (MLV) Env interacts with Pit-2 cellular receptor (Miller *et al.*, 1994; Van Zeijl *et al.*, 1994), which is present on mouse and human cells. In contrast, Moloney (ecotropic) MLV Env interacts with CAT-I cellular receptor (Albritton *et al.*, 1989), which is present on mouse cells but not human cells. Hence, vectors incorporating amphotropic envelope proteins can infect human cells, whereas ecotropic viruses cannot.

Retroviral gene delivery can be controlled by engineering retroviral envelope proteins. WO 94/06920 discloses a method whereby non-viral polypeptides are fused to the N-terminus of Env and thereby displayed on the surface of a virus particle. Using this method, single-chain antibody fragments to hapten (Russell *et al.*, 1993), CD3, and four colonic carcinoma cell antigens (Ager *et al.*, 1996) have been displayed and shown to bind specifically to their antigen-target. However, gene delivery does not necessarily result

from this interaction. The cellular growth factors epidermal growth factor (EGF), stem cell factor (SCF) and insulin-like growth factor I (IGF-I) have also been displayed successfully (Cosset *et al.*, 1995; Fielding *et al.*, 1998; Chadwick *et al.*, 1999). The display of these ligands inhibits envelope-receptor mediated gene delivery when ligand receptors are present on the target cells. Gene delivery is impaired by binding of the chimeric envelopes to ligand receptors that do not support virus entry and sequester virus away from the virus receptor.

Gene delivery can also be impaired by display of a polypeptide which can form an 'oligomeric cap' on the trimeric viral glycoprotein (W0960456; Morling *et al.*, 1997). The oligomeric cap forms by intermolecular association between the heterologous polypeptides displayed on different Env subunits within the trimeric envelope glycoprotein (Figure 1). The C-terminal extracellular domain of CD40 ligand has been shown to form such an oligomeric cap when displayed on trimeric viral glycoproteins. CD40 ligand is a homotrimer, and hence exhibits the same stoichiometry of association as the envelope glycoprotein itself. CD40 ligand display significantly reduced envelope-protein mediated gene transfer to target cells, by inhibiting binding of Env to its receptor and likely also by inhibiting subsequent fusion triggering, which requires the dissociation of trimeric Env into its subunits. The display of trimeric 'leucine zipper' polypeptides effects a similar phenotype via the formation of an 'oligomeric cap' (Morling *et al.*, 1997). This study demonstrated that displayed oligomeric proteins can be detrimental to incorporation of Env into vector particles. The effect is probably due to rapid oligomerization of the displayed protein prior to Env folding in the endoplasmic reticulum.

Thus, gene delivery can be impaired *via* the two mechanisms of receptor-mediated sequestration and 'envelope protein' blockade. The reversal of this phenotype enables those skilled in the art to control the ability of a viral vector to infect a particular cell type. Such reversal can be effected in two ways. First, in the case of viruses displaying ligands, any reduction in available ligand receptor binding sites will result in increased gene delivery, since more chimeric Env will be free to bind to virus receptor. Typically, this can be achieved by addition of an agonist, such as free ligand (Cosset *et al.*, 1995;

Fielding *et al.*, 1998; Chadwick *et al.*, 1999). Such a system can be used as the basis of an assay for substances that interact with ligand receptors (WO 97/03357).

Second, gene delivery can be restored upon removal of any displayed heterologous polypeptide. The displayed domain can be removed by protease cleavage of a substrate sequence situated between the displayed domain and Env. This mechanism can be utilized to generate vectors that are activated in the presence of a specific protease (WO 97/12048). Such vectors can be targeted to infect particular cell types, such as cancer cells expressing elevated levels of matrix metalloproteinases (Peng *et al.*, 1997), for use in gene therapy protocols.

Certain ligands, which would be advantageously used in a display system, are oligomeric. For example, cysteine knot growth factors are biologically active as dimers cross-linked by disulfide bonds (Sun & Davies, 1995). Display of oligomeric ligands, however, can present problems not associated with display of monomeric ligands, including reduced incorporation of the chimeric envelope proteins into vector particles. In addition, since the displayed proteins are both ligands and oligomers, gene delivery could be undesirably impaired by both receptor-mediated sequestration and envelope protein blockade.

Thus, there is a need in the art for techniques for displaying functional oligomeric ligands so that these ligands can bind to specific cell surface receptors.

Summary of the Invention

It is an object of the present invention to provide compositions for displaying functional homo-oligomeric ligands and methods of using the displayed homo-oligomeric ligands in screening assays for new drugs and in methods of specifically delivering a desired substance to a target cell. These and other objects of the invention are provided by one or more of the embodiments described below.

In a first aspect the invention provides a recombinant fusion protein comprising a first polypeptide and a second polypeptide fused to each other via peptide bonding, wherein the first polypeptide comprises first and second domains in a continuous polypeptide chain

which self-associate to form a homo-oligomeric ligand which has the ability to bind to a receptor. Preferably the first polypeptide is a dimer or a trimer.

In a second aspect the invention provides a recombinant fusion protein comprising a first polypeptide and a second polypeptide fused to each other via peptide bonding, wherein the first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form an oligomeric ligand, a said domain comprises a disulfide bonded growth factor, and wherein said oligomeric ligand has the ability to bind to a receptor.

In preferred embodiments of the first or second aspects of the invention, the first polypeptide comprises at least two subunits of a cysteine knot growth factor. The second polypeptide typically comprises a substantially intact viral envelope protein that binds to a cell-surface receptor and mediates fusion between a cellular membrane and a viral membrane, *i.e.*, enhances fusion above the level of fusion which occurs in the absence of the substantially intact viral envelope protein. The at least two subunits of the cysteine knot growth factor advantageously associate to form a cysteine knot growth factor which binds to a cysteine knot growth factor receptor.

In a further aspect the invention provides a viral display package comprising a recombinant fusion protein which comprises a first polypeptide and a second polypeptide fused to each other via peptide bonding. The first polypeptide may comprise first and second domains in a continuous polypeptide chain which self-associate to form an homo-oligomeric ligand which has the ability to bind to a receptor. Alternatively, the first and/or second domain may comprise a disulfide bonded growth factor and first and second domains, which are a continuous polypeptide chain, self-associate as an oligomeric ligand. The second polypeptide is preferably anchored to a retroviral membrane.

In yet another aspect the invention provides a library of viral display packages, in which each of a plurality of viral display packages comprises a recombinant fusion protein. The recombinant fusion protein comprises a first polypeptide and a second polypeptide fused to each other via peptide bonding. The first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form an homo-oligomeric ligand

which has the ability to bind to a receptor. The second polypeptide is anchored to a retroviral membrane.

In a further aspect the invention provides an isolated polynucleotide which encodes a recombinant fusion protein which comprises a first polypeptide and a second polypeptide fused to each other via peptide bonding. The first polypeptide comprises at least two subunits of a cysteine knot growth factor with a characteristic PDGF-like family signature selected from the group consisting of PDGF and VEGF. The second polypeptide comprises a substantially intact viral envelope protein which enhances fusion between a cellular membrane and a viral membrane. The viral envelope protein is a mammalian type C retrovirus envelope protein and can be selected from the group consisting of a 4070A envelope protein, a Moloney MLV envelope protein, and a gibbon ape leukemia virus (GALV). The at least two subunits of the cysteine knot growth factor associate to form a cysteine knot growth factor which binds to a cysteine knot growth factor receptor.

In another aspect the invention provides a method of screening a test substance for the ability to modulate binding of an homo-oligomeric ligand to a receptor, such as by preventing or reducing the ligand-receptor interaction. A lipid enveloped particle and a target cell are contacted with a test substance. The lipid enveloped particle comprises (a) a transferable label and (b) a recombinant fusion protein present on the outer surface of the lipid enveloped particle and comprising a first and a second polypeptide. The first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form an homo-oligomeric ligand which has the ability to bind to a receptor present on the outer surface of the target cell. The amount of the transferable label in the target cell is measured in the presence of the test substance. An increase in the amount of transferable label in the target cell relative to the amount of transferable label in a target cell in the absence of the test substance indicates reduction or inhibition of binding of the homo-oligomeric ligand to the receptor.

In still a further aspect the invention provides method of targeting a transferable label to a target cell, comprising the step of:

contacting a target cell with a lipid enveloped particle, wherein the lipid enveloped particle comprises (a) a transferable label and (b) a recombinant fusion protein present on the outer surface of the lipid enveloped particle and comprising a first and a second polypeptide, wherein the first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form an oligomeric ligand which has the ability to bind to a receptor present on the outer surface of the target cell, whereby a lipid enveloped particle-target cell complex is formed.

In yet a further aspect the invention provides a method of delivering a transferable label to a target cell, comprising the steps of:

contacting a target cell with a lipid enveloped particle, wherein the lipid enveloped particle comprises

(a) a transferable label; and

(b) a recombinant recombinant fusion protein present on the outer surface of the lipid enveloped particle and comprising a first and a second polypeptide separated by a protease recognition site, wherein the first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form an oligomeric ligand which has the ability to bind to a receptor present on the outer surface of the target cell, whereby a lipid enveloped particle-target cell complex is formed;

and contacting the lipid enveloped particle-target cell complex with a protease which recognizes the protease recognition site, whereby the first polypeptide is cleaved from the second polypeptide and the transferable label is transferred to the target cell.

Yet another embodiment of the invention is a method of screening a test substance for the ability to prevent binding of an oligomeric ligand to a receptor. A target cell is contacted with a viral particle. The viral particle comprises (a) a transferable label and (b) a recombinant fusion protein present on the outer surface of the viral particle and comprising a first and a second polypeptide. The first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form an

oligomeric ligand which has the ability to bind to a receptor present on the outer surface of the target cell. The amount of transferable label in the target cell is measured in the presence of the test substance. An increase in the amount of transferable label in the target cell relative to the amount of transferable label in a target cell in the absence of the test substance indicates inhibition of binding of the oligomeric ligand to the receptor.

Still another embodiment of the invention is a method of screening a test substance for the ability to dissociate an oligomeric ligand into its constituent subunits, (such that it has reduced capacity to bind to a receptor) the method comprising the steps of:

contacting a target cell with a viral particle, wherein the viral particle comprises (a) a transferable label and (b) a recombinant fusion protein present on the outer surface of the viral particle and comprising a first and a second polypeptide, wherein the first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form an oligomeric ligand which has the ability to bind to a receptor present on the outer surface of the target cell; and measuring the amount of transferable label in the target cell in the presence of the test substance, wherein an increase in the amount of transferable label in the target cell relative to the amount of transferable label in a target cell in the absence of the test substance indicates dissociation of an oligomeric ligand into its constituent subunits.

Thus, the present invention provides an innovative approach to the effective retroviral display of functional oligomeric ligands, particularly cysteine knot growth factors.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram which shows the display of a trimeric murine leukemia virus envelope polypeptide which forms an "oligomeric cap" on the envelope protein.

Figure 2 is a diagram which shows the structure of VEGF, showing the intermolecular disulfide bonds and the antiparallel arrangement of the cysteine knot domains.

Figure 3A is a diagram which shows display of a single CKGF domain per Env subunit. Difulfide bonds form between domains displayed on adjacent Env subunits (intermolecular).

Figure 3B is a diagram which shows display of CKGF single chain dimers. Disulfide bonds form between CKGF domains within each single-chain dimer (intramolecular), such that one dimer is displayed per Env subunit.

Figure 4A is a diagram which shows display of one CKGF domain.

Figure 4B is a diagram which shows display of two CKGF domains as single-chain molecules.

Figure 5 is an immunoblot of retroviral envelope proteins. Lane 1, PDGFNG4SA; lane 2, PDGFNXA; lane 3, ScPDGFNG4SA; lane 4, ScPDGFNXA; lane 5, unmodified 4070A envelope protein.

Figure 6 is an immunoblot of chimeric retroviral envelope proteins following non-reducing SDS-PAGE. Lane 1, PDGFNG4SA; lane 2, PDGFNXA; lane 3, ScPDGFNG4SA; lane 4, ScPDGFNXA; lane 5, unmodified 4070A envelope protein.

Figure 7 presents immunoblots of chimeric retroviral envelope proteins with no prior treatment with Factor Xa protease (-) or with treatment with Factor Xa protease for 90 minutes prior to loading (+) following non-reducing SDS-PAGE. Lane 1, PDGFNG4SA; lane 2, PDGFNXA; lane 3, unmodified 4070A Env; lane 4, ScPDGFNG4SA; lane 5, ScPDGFNXA.

Figure 8 is a graph which shows infection of NIH 3T3 cells with ScPDGFNG4SA vectors in the presence of increasing concentrations of PDGF-BB free ligand.

Figure 9 is an immunoblot of retroviral envelope proteins. Lane 1, VEGFNG4SA; lane 2, VEGFNXA; lane 3, ScVEGFNG4SA; lane 4, ScVEGFNXA; lane 5, unmodified 4070A envelope protein.

Figure 10 presents immunoblots of chimeric retroviral envelope proteins with no prior treatment with Factor Xa protease (-) or with treatment with Factor Xa protease for 90 minutes prior to loading (+) following non-reducing SDS-PAGE. Lane 1, VEGFNG4SA; lane 2, VEGFNXA; lane 3, ScVEGFNG4SA; lane 4, ScVEGFNXA; lane 5, unmodified 4070A envelope protein.

DETAILED DESCRIPTION

The present invention permits the display of oligomeric ligands, such as dimeric or trimeric ligands, as recombinant fusion proteins, in a conformation that permits binding to a receptor for the oligomeric ligand. The displayed oligomeric ligands can be used, for example, to target a transferable label to a target cell bearing on its surface a receptor for the oligomeric ligand. Such targeting can be used for a variety of purposes, such as drug screening and delivery of transferable labels, including genes.

Oligomeric Ligands Useful According to the Invention

As used herein, the phrase "homo-oligomeric ligand" means at least two copies of a substantially identical ligand. "Substantially identical" means either identical, i.e., having identical amino acid sequences, or it means that the two ligands are at least 80%, preferably 90%, more preferably at least 95-98% identical in amino acid sequence over the entire length when the two molecules are aligned, and the two ligands self-associate to form a homo-oligomeric ligand that has ability to bind to a receptor.

Self-association involves interaction between the two or more substantially identical ligands via covalent and/or non-covalent bonds. Typically the dissociation constant (Kd) of the self-associated homo-oligomeric complex is 100M or less, preferably 10M or less, more preferably 1M or less.

There are 3 types of homoligomeric ligands: naturally homodimeric or trimeric ligands, in which subunits are disulfide bonded; ligands that are naturally dimeric or trimeric in which there are no disulfide bonds so that the ligands are free to dissociate (e.g., stem cell factor); and naturally monomeric ligands where the binding properties may be enhanced by driving the ligand to assume a multimeric form (e.g., EGF). For the

latter, naturally monomeric ligands, by fusing monomers into dimers, trimers, etc., the receptor binding affinity is contemplated to increase, e.g., by at least 10-fold, 50-fold, 100-fold, or more. Osteoprotegerin (OPG-1), for example, functions as a homodimer, circulates as a monomer, and dimerizes upon binding to its receptor.

As used herein, the phrase "hetero-oligomeric ligand" refers to at least two oligomers which are substantially non-identical in sequence and binding specificity. That is, the hetero-oligomers have less than 79% sequence identity, preferably less than 70%, more preferably less than 60%, most preferably less than 50% or 40% over the entire length of the molecules, and also have different binding specificities in the hetero-oligomeric form in comparison to another hetero-oligomer having a different set of ligands.

Oligomeric ligands, which can be displayed according to the invention, are typically dimeric or trimeric but can comprise four or more subunits. Growth factors, particularly cysteine knot growth factors (CKGFs), are particularly well suited for such display. CKGF polypeptides are characterized by a common three-dimensional 'cysteine knot' configuration. Cysteine knots are the result of an unusual arrangement of six cysteine residues, three cysteine residues on each polypeptide subunit of the dimer. The knot consists of bonds 1-4, *2-5* and the intervening sequence forming a ring, through which disulfide bond 3-6 passes (Figure 2). In the case of the PDGF-like family, the native protein is a dimer of two domains in an anti-parallel orientation. The subunits may be identical (forming a homodimer) or non-identical (forming a heterodimer).

Members of the CKGF family of proteins, including PDGF, VEGF, NGF and TGF- β-2, are extremely attractive candidates for retroviral display. In particular, PDGF and VEGF are involved in the process of angiogenesis, whereby new blood vessels are formed from existing vasculature. This process is implicated in tumor growth, because metastatic tumors must recruit a new blood supply before they can increase beyond a few millimeters in diameter (Ausprunk & Folkman, 1977). Hence, PDGF and VEGF receptors are attractive targets for anti-cancer drugs. Retroviral display of functional PDGF and VEGF would facilitate the generation of a cellular drug-screening assay, as

described in WO 97/03357, and vectors capable of delivering genes to vascular endothelial cells following proteolytic activation for use in gene therapy protocols.

Dimeric ligands which can be displayed according to the invention include members of the PDGF family (including PDGF, PDGF-related transforming protein P28-SIS, VEGF, VEGF B, VEGF C, VEGF homolog, PLGF, PLGF-1, PLGF-2, VEGF D, and VEGF E), TGF-β family, glycoprotein hormones/gonadotropins, trefoil polypeptide family, NGF family, insulin family, HBGF/FGF family, intercrine alpha/small cytokine CXC/chemokine CXC family, small cytokine CC/intercrine β family, and interleukin families, and OPGL (osteoprotegerin, Kong et al., 1999). Trimeric ligands which can be displayed include members of the tumor necrosis factor family and macrophage migration inhibitory factor (MIG) family. Amino acid sequences and nucleotide sequences encoding these proteins can be obtained from various databases, such as GenBank, SWISS-PROT, EMBL DNA sequence database, PIR protein database, Vecbase, or GenPept. Ligands which comprise a PDGF-like family signature are particularly preferred. Such ligands include the consensus sequence P-[PS]-C-V-X-X-X-R-C-[GSTA]-G-C-C (SEQ ID NO: 15), where the four cysteine residues are involved in disulfide bonds.

Oligomeric ligands are displayed as single chain polypeptides within a recombinant fusion protein. Recombinant fusion proteins of the invention are not naturally occurring but can be produced using recombinant DNA technology or can be synthesized using standard protein synthesis techniques. Recombinant fusion proteins comprise a first and a second polypeptide fused to each other via peptide bonding. The first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form the oligomeric ligand. One or more additional domains can be included to form trimeric or other oligomeric ligands. Preferably, a nucleic acid sequence encoding the first polypeptide is linked, without disruption of the translational reading frame, to nucleic acid sequences coding for the second polypeptide.

Cell-Surface Receptor Binding Polypeptides Useful According to the Invention

The second polypeptide is preferably a polypeptide that binds to a cell-surface virus receptor and mediates fusion between a cellular membrane and a lipid envelope, such as a

synthetic lipid envelope or a viral, particularly a retroviral, membrane. Viral glycoproteins, including mammalian type-C retroviral envelope proteins such as gibbon ape leukemia virus (GALV) envelope protein, 4070A envelope protein, and Moloney MLV envelope protein, are particularly useful as the second polypeptide. It is important that the viral glycoprotein is substantially intact, *i.e.* retains all its domains, to conserve post-translational processing, oligomerization, viral incorporation, and fusogenic activities. However, certain alterations, such as mutations, deletions, or additions, can be made to the glycoprotein which do not significantly affect these functions, and glycoproteins with such modifications are considered substantially intact.

Preferably, the first polypeptide is fused close to the terminus of the mature envelope glycoprotein so that folding of the domains of the first polypeptide into a functional oligomeric ligand is not significantly disturbed. Fusions within 30, 20, or 10 amino acids of the N terminus can be used, although fusions nearest the N-terminus are generally optimal for preserving infection competence of the recombinant fusion protein. For example, fusions at amino acid 1 are preferred over fusions between amino acids 6 and 7. If desired, linker sequences, which can function as "hinges," comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 or more contiguous amno acids can be included between any two of the domains of the first polypeptide to facilitate folding of the first polypeptide into the conformation similar to that of the native oligomeric ligand. Amino acid sequences suitable for use as linkers between any two domains can be empirically determined using routine screening methods or can be predicted based on the known abilities of particular amino acid sequences to permit folding of two adjacent polypeptide domains *(see, e.g.,* Alfthan *et al.,* 1995). A suitable linker sequence for linking two VEGF subunits, for example, is Ala-Ala-Gly-Gly-Gly-Gly-Gly-Ser (SEQ ID NO:7).

Optionally, a protease recognition site is present between the first and second polypeptides. Such protease recognition sites can be used to control fusion of a lipid enveloped particle, such as a viral particle, with a target cell membrane, thereby controlling delivery of a transferable label or gene from the particle to the target cell. A variety of protease recognition sites are available for use in recombinant fusion proteins. For example, a Factor Xa site or an elastase recognition sites, such as N-Ac-Ala-Ala-

DOPE, Ala-Ala-Pro-Val (SEQ ID NO:10), and MeO-Suc-Ala-Ala-Pro-Val-pNA (SEQ ID NO:11), can be incorporated into the recombinant fusion protein (Zimmerman and Ashe, 1977; Nakajima *et al.*, 1979). Cathepsin G recognition sites, such as MeO-Suc-Ala-Ala-ProMet-pNA (SEQ ID NO:12) (Zimmerman and Ashe, 1977), Suc-Ala-Ala-Pro-Phe-pNA (SEQ ID NO:13), and Suc-Ala-Ala-Pro-Lys-pNA (SEQ ID NO:14) (Polanowska *et al.*, 1998), can also be used.

The second polypeptide of the recombinant fusion protein can be anchored to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, or particles such as beads, including but not limited to latex, polystyrene, or glass beads. In preferred embodiments, the solid support is a lipid enveloped particle, biological membrane, or viral capsid. Lipid enveloped particles can be used to enclose a transferable label and include, but are not limited to, synthetic lipid bilayers, native or recombinantly modified enveloped viral particles, and prokaryotic and eukaryotic cells. Biological membranes include the envelopes of viruses, including retroviruses, as well as the cell membranes of prokaryotic and eukaryotic cells. Useful viruses include members of the adenovirus, togavirus, rhabdovirus, and retrovirus families, as well as enveloped viruses such as paramyxovirus and orthomyxovirus. Amphotropic viruses, capable of infecting eukaryotic cells, are particularly useful.

Polynucleotides Encoding Recombinant Fusion Proteins According to the Invention

The invention also provides polynucleotides which encode recombinant fusion proteins of the invention. Polynucleotides of the invention contain less than a whole chromosome and can be single- or double-stranded. Preferably the polynucleotides will comprise DNA (which may be genomic or cDNA), but may also comprise RNA and may optionally include synthetic (non-naturally occurring) bases. Preferably the polynucleotides are isolated free of other cellular components, such as membrane components, proteins, and lipids. They can be made by a cell and isolated, or synthesized in the laboratory using an amplification method such as PCR or using an automatic

synthesizer. Methods for purifying and isolating DNA are routine and are known in the art.

In one embodiment, the polynucleotide encodes a recombinant fusion protein in which the first polypeptide comprises subunits of either PDGF or VEGF and the second polypeptide comprises a mammalian type-C retrovirus envelope protein, such as a Moloney MLV envelope protein, a 4070A envelope protein, or a GALV envelope protein. The amino acid sequences of these proteins are known in the art. Any nucleotide sequence which encodes such recombinant fusion proteins is encompassed within the invention.

Viral Display Packages

A viral display package is a virus which has been genetically engineered to display on its surface a recombinant fusion protein of the invention. Viral display packages comprise recombinant fusion proteins of the invention and are formed when the second polypeptide is anchored to a viral membrane. Viral display packages can include multiple recombinant fusion proteins; if desired, the recombinant fusion proteins which each comprise different oligomenc ligand domains can be incorporated in a viral display package, to provide a viral display package with multiple target specificity. Viral display packages are preferably based on viruses which can infect eukaryotic cells, such as MLV or other C type retroviruses, vesicular stomatitis virus, influenza virus, Semliki Forest virus, Sindbis virus, Sendai virus, or adenoviruses.

Libraries

Libraries of viral display packages can also be constructed. Such libraries comprise at least two viral display packages, which may or may not have the same target specificity. Unique restriction sites can be inserted between codons for the second polypeptide for generation of vectors to generate libraries of replication-competent viruses displaying fusion proteins of the invention. For example, unique restriction sites *Sfi*I and *NotI* could be inserted between the codons for the 6th and 7th amino acids of the mature envelope protein in a full-length infectious molecular clone of MoMLV (Shoemaker *et al.*, 1980), without disruption of the translational reading frame. After removing the *Sfi*I site

in the gag coding region of MoMLV (Shinnick *et al.*, 1981), the vectors can be used to generate libraries of replication-competent viruses displaying fusion proteins of the invention. Any PCR-generated library of DNA fragments encoding the fusion proteins, for example, could be cloned into this site. Clearly, for any given second polypeptide there are alternative combinations of noncomplementary restriction sites which would serve equally well and in some cases a single restriction site may be sufficient.

The generation of a diverse library of recombinant retroviruses has been demonstrated previously by transient transfection of retroviral plasmids into retroviral packaging cells (Murphy and Efstratiadis, 1987). The library size is limited by the efficiency of plasmid transfection, first into *E. coli* for growth and purification of the plasmid ligation product and second into the retroviral packaging cells for generation of the retroviral genetic display library. Employing recently developed and highly efficient methods of gene delivery to mammalian cells (Curiel *et al.*, 1992), we estimate that a library size of 10^8 viral display packages should be possible.

Screening Methods

Recombinant fusion proteins, including those present on the surface of lipid enveloped particles and viral particles, can be used in methods of screening a test substance for the ability to prevent binding of an oligomeric ligand to its receptor. In the screening methods of the invention, the test substance may be in free form and/or may be pre-bound to the receptor on the surface of the target cell. Test substances which can be screened can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. Test substances can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, or can be produced recombinantly or synthesized by chemical methods known in the art.

Screening methods can be carried out *in vivo* or *in vitro*. In one embodiment, a lipid enveloped particle, such as a viral particle, and a target cell are contacted with a test substance. The viral or other lipid enveloped particle comprises both a transferable label and a recombinant fusion protein of the invention. The recombinant fusion protein, by

virtue of the displayed oligomeric ligand present in the first polypeptide of the fusion protein, is used to specifically bind the lipid enveloped particle to the target cell, to form a lipid enveloped particle-target cell complex.

Transferable Labels and Cell Targeting

The transferable label can be any label whose presence can be detected in the target cell upon fusion of the lipid enveloped particle and the target cell membrane, such as a radiolabel or a nonisotopic label, for example a chemiluminescent, fluorescent, or enzymatic label. The transferable label can be a selectable marker, such as an antibiotic-resistance gene or the like, which facilitates identification and selection of target cells which have been infected by a lipid enveloped particle. The transferable label can also be a reporter gene which encodes a detectable product, such as ß-galactosidase, luciferase, ß-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), or chloramphenicol acetyltransferase (CAT). Many such genes are known in the art.

Transferable labels may also include any protein product whose delivery to a target cell is desired for gene therapy purposes, such as alpha-1 antitrypsin, globin proteins, Factor VIII or Factor IX proteins, growth factors, cytokines, or LDL receptors. Such transferable labels can be detected, for example, using immunochemical techniques employing antibodies which specifically bind to the protein product. Other transferable labels include antisense transcripts or ribozymes, which can be detected using specific oligonucleotide probes. Other pharmacologically active substances can be used as transferable labels.

A receptor for the particular oligomeric ligand displayed by the lipid enveloped particle is present on the outer surface of the target cell, which permits specific targeting of the lipid enveloped particle to the target cell. Receptors which can be targeted include, but are not limited to, receptors for oligomeric growth factors, such as CKGFs. Such receptors include receptors for members of the PDGF family (including PDGF, PDGF related transforming protein P28-SIS, VEGF, VEGF B, VEGF C, VEGF homolog,

PLGF, PLGF-1, PLGF-2, VEGF D, and VEGF E), TGF-β family, glycoprotein hormones/gonadotropins, trefoil polypeptide family, NGF family, insulin family, HBGF/FGF family, intercrine alpha/small cytokine CXC/chemokine CXC family, small cytokine CC/intercrine β family, and interleukin families, as well as receptors for members of the tumor necrosis factor family and macrophage migration inhibitory factor (MIG) family. The target cell can naturally comprise the receptor or the receptor can be introduced into the target cell, for example using a recombinant nucleic acid construct encoding the receptor. Methods of transfecting nucleic acid constructs into cells are well known and include, but are not limited to, transfection with naked or encapsulated nucleic acids, cellular fusion, protoplast fusion, viral infection, and electroporation.

The target cell can be isolated from a eukaryote, preferably a mammal, more preferably a human, and placed in tissue culture. Established cell lines can also be used to provide the target cell. Alternatively, the target cell can be present in a mammalian body, such as a rat, mouse, guinea pig, hamster, rabbit, goat, sheep, or human body.

The amount of transferable label in the target cell is measured in the presence of the test substance. Measurement can be by any method suitable for detecting the particular transferable label used, including biochemical, immunological, or visual detection methods. Expression of a gene can also be determined by detecting its mRNA, for example using Northern or dot blots or *in situ* hybridization. A test substance which increases the amount of transferable label in the target cell relative to the amount of transferable label in a target cell in the absence of the test substance indicates inhibition of binding of the oligomeric ligand to the receptor. The increase in the amount of transferable label can be determined qualitatively or quantitatively, for example by reference to a standard curve. Preferably, the test substance increases the amount of transferable label in the target cell by at least 25%, 50%, 100%, or 2-fold, 5-fold, 10-fold, or even up to 1,000-fold. Generally, a test substance that inhibits the binding of the oligomeric ligand to the receptor will be considered inhibitory if it increases the amount of label transferred by at least about 25%.

Recombinant fusion proteins that include single chain polypeptides when present on the surface of a lipid enveloped particle, such as a viral particle, can also be used to target a transferable label to a target cell, as described above, or to deliver the transferable label to the target cell. Delivery of the transferable label may be for therapeutic purposes or may be used to label a particular population of target cells, for example for subsequent isolation of the population from non-target cells.

For delivery of the transferable label, the recombinant fusion protein can comprise a protease recognition site located between the first and second polypeptides. After formation of the lipid enveloped particle-target cell complex as described above, the complex is contacted with a protease which recognizes the protease recognition site. The protease can be normally present at the location of the target cell, or can be introduced, for example by injection at the site of the target cell in a mammalian body or by addition of the protease to a tissue culture medium. The protease recognition site is then cleaved, which releases the first polypeptide comprising the oligomeric ligand now bound to its receptor. The second polypeptide is then able to effect fusion between the lipid enveloped particle and the target cell membrane, thereby permitting delivery of the transferable label to the target cell.

The invention also provides a method of screening a test substance for the ability to dissociate an oligomeric ligand into its constituent subunits, such that it has reduced capacity to bind to a receptor. A target cell is contacted with a viral particle. The viral particle comprises (a) a transferable label and (b) a recombinant fusion protein present on the outer surface of the viral particle and comprising a first and a second polypeptide. The first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form an oligomeric ligand which has the ability to bind to a receptor present on the outer surface of the target cell. The amount of transferable label in the target cell is measured in the presence of the test substance. An increase in the amount of transferable label in the target cell relative to the amount of transferable label in a target cell in the absence of the test substance indicates dissociation of an oligomeric ligand into its constituent subunits.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided herein for purposes of illustration only and are not intended to limit the scope of the invention. All references cited in this disclosure are expressly incorporated herein.

EXAMPLE 1

Methods used in the examples below

PDGF Plasmid construction. The constructs PDGFNG4SMo, PDGFNXMo, PDGFNG4SA, and PDGFNXA were generated as follows. Primers *Sal*IPDGFBF 5'(5'-CCAGCTGTCGACTAGCCTGGGTTCCCTGACCATTGC-3' SEQ ID NO:1) and PDGFNotIR3': (5'-ATCGATGCGGCCGCGGTCACAGGTCGTGCAGCTGCCACTGTCTC-3'; SEQ ID NO:2) were used to amplify PDGF from pPDGFB plasmid (ATCC 57050) and introduce *Sal*I and *Not*I restriction sites at the 5' and 3' ends of the coding region respectively. The PCR product was subsequently digested with *Sal*I and *Not*I restriction endonucleases and ligated into *Sal*I/*Not*I-digested IGFNG4SMo, IGFNXMo, IGFNG4SA, and IGFNXA (Chadwick *et al*, 1999) generating PDGFNG4SMo, PDGFNXMo, PDGFNG4SA, and PDGFNXA, respectively. To generate the constructs ScPDGFNG4SMo, ScPDGFNXMo, ScPDGFNG4SA and ScPDGFNIXA a PDGF PCR product was produced using the primers PDGFBNotIR and EagIPDGFBF5': (5'CGATATGCGGCCGGA-GGTGGAGGCGGTTCAAGCCTGGGTTCCCTGACCATTGC-3'; SEQ ID NO:3) which amplified PDGF from pPDGFB and introduced in-frame *Eag*I and *Not*I sites at the 5' and 3' ends of the coding sequence. This PCR product was cut with *Eag*I and ligated into *Not*I digested PDGFNG4SMo, PDGFNXMo, PDGFNG4SA and PDGFNXA. Plasmid orientation was checked using a *Sal*I/*Not*I digest. The ligation resulted in the production of ScPDGFNG4SMo, ScPDGFNXMo, ScPDGFNG4SA and ScPDGFNXA. DNA sequencing confirmed that all constructs included the correct sequences.

VEGF plasmid construction. Constructs VEGF.NG4SMo, VEGF.NXMo, VEGF.NG4SA, and VEGF.NXA were generated by PCR cloning VEGF into

IGF.NG4SMo, IGF.NXMo, IGF.NG4SA, and IGF.NXA (Chadwick *et al*, 1999) backbone DNA, respectively. Primers *Sal*I-VEGF-For (5' - CCAGCTGTCGACTGGGCAGAATCATCACGAAGTGG-3'; SEQ ID NO:4) and VEGF-*Not*I-Rev (5'-ATCGATGCGGCCGCTG-TTGTTGGTCTGCATTCACATTTGTTGT-3'; SEQ ID NO:5) were used to generate an amplification product from pVEGF (a plasmid clone of $VEGF_{121}$ cDNA) (Cohen *et al*, 1995) encoding the subunit of the VEGF receptor binding domain with introduced in-frame *Sal*I and *Not*I restriction sites. The amplification product was digested with *Sal*I and *Not*I restriction endonucleases ready for ligation. Constructs IGF.NG4SMo, IGF.NXMo, IGF.NG4SA, and IGF.NXA DNA were double digested with *Sal*I and *Not*I restriction endonucleases, and the approximately 10 kb *Sal*I-backbone-*Not*I DNA was separated from the approximately 220 bp *Sal*I-IGF-*Not*I insert DNA by agarose gel electrophoresis. The *Sal*I-backbone-*Not*I DNA was gel purified and the *Sal*I-VEGF-*Not*I insert DNA directionally cloned into the four backbone DNAs to generate VEGF.NG4SMo, VEGF.NXMo, VEGF.NG4SA, and VEGF.NXA.

Constructs scVEGF.NG4SMo, scVEGF.NXMo, scVEGF.NG4SA, and scVEGF.NXA were generated by PCR cloning VEGF into VEGF.NG4SMo, VEGF.NXMo, VEGF.NG4SA, and VEGF.NXA backbone DNA, respectively. Primers *Eag*I-VEGF-For:(5'-CGATATGCGGCCGGAGGTGGAGGCGGTTC-AGGGCAGAATCATCACGAAGTGG-3'; SEQ ID NO:6) and VEGF-*Not*I-Rev were used to generate an amplification product from pVEGF encoding the same subunit of the VEGF receptor binding domain but with introduced in-frame *Eag*I and *Not*I restriction sites. It also introduced an Ala-Ala-Gly-Gly-Gly-Gly-Gly-Ser (SEQ ID NO:7) hinge between the two VEGF subunits. The amplification product was digested with *Eag*I restriction endonuclease ready for ligation. Constructs VEGF.NG4SMo, VEGF.NXMo, VEGF.NG4SA and VEGF.NXA were digested with *Not*I restriction endonuclease and the linearized plasmid DNA purified. The *Eag*I-VEGF-*Eag*I insert DNA was non-directionally cloned into the *Not*I-digested backbone DNA. A clone with the insert VEGF DNA in the same coding orientation as the VEGF sequence in the backbone DNA was selected after screening of the resultant clones by their *Sal*I/*Not*I restriction pattern.

Nucleotide sequencing of both strands of the DNA between the *SalI* and *NotI* restriction sites was performed for all constructs to confirm that no polymerase errors were introduced into the insert nucleotide sequence by PCR.

Production of viruses. The chimeric envelope expression constructs were transfected into TELCeB6 cells by superfection. Stably transfected cells were transferred into medium containing 50 μg/ml phloemycin and 6 μg/ml blasticidin. Pooled colonies of cells were allowed to reach confluency, and viral supernatant harvested following overnight incubation in serum free DMEM (for infections) or DMEM/2% fetal calf serum (for immunoblots). All supernatants were filtered (0.45 μm) before use.

Immunoblots. Pelleted virus was obtained from filtered (0.45 μm) viral supernatant by ultracentrifugation at 30,000 rpm in a SW4O Ti rotor (Beckman) for one hour at 4°C. The supernatant was discarded and pelleted virus resuspended in 100 μl ice-cold PBS. Aliquots (10 μl) were mixed with loading buffer containing SDS, the samples boiled and subjected to SDS-PAGE on a 10% (w/v) polyacrylamide gel. The proteins were subsequently transferred onto nitrocellulose and the envelope protein was detected using specific goat antibodies raised against Rauscher leukemia virus gp 70-SU envelope protein. Blots were developed with horseradish peroxidase-conjugated rabbit anti-goat antibodies and enhanced chemiluminescence kit. In factor Xa cleavage experiments aliquots were incubated for 90 minutes at 37 °C in the presence or in the absence of 4 μg/ml factor Xa protease before subjection to SDS-PAGE. In non-reducing gels DTT (200 mM) was removed from the SDS-loading gel before addition to aliquots.

Infections. NIH 3T3 cells were seeded at 5 x 10^4 cells/well in 24-well plates and incubated at 37 °C overnight. In the ligand competition experiment the cells to be infected were additionally incubated at 37 °C for 30 minutes in the absence of ligand or in the presence of different concentrations of ligand prior to infection with virus. Following these incubation steps, 10 μl of filtered (0.45 μ m) viral supernatant was diluted in serum-free medium to make up a total volume of 500 μl. The infection was then allowed to proceed for 6 hours at 37 °C. The cells were then washed once with PBS and supplied with fresh DMEM/10% foetal bovine serum. After 24-48 hours, when the cells had grown to

confluency, X-gal (5-bromo-4-chloro-3-indolyl β-D-galactosidase) staining was performed, to determine the number of infective events by detection of β-galactosidase activity.

EXAMPLE 2

PDGF display

An array of envelope proteins displaying PDGF were constructed. Such constructs consisted of the envelope protein itself (Moloney or 4070A), a linker peptide (Ala-Ala-Ala-Gly-Gly-Gly-Ser (SEQ ID NO:8) or the factor Xa site Ala-Ala-Ala-Ile-Glu-Gly-Arg (SEQ ID NO:9) and the displayed domain (PDGF-B single domain or single-chain dimer) (see Figure 4). The chimeric and unmodified Moloney and 4070A envelope expression plasmids were transfected into TELCeB6 viral producer cells.

Immunoblotting of pelleted virus was performed to determine whether the chimeric envelope proteins were incorporated into vector particles. Figure 5 shows an immunoblot for the chimeric constructs PDGFNG4SA, PDGFNXA, ScPDGFNG4SA and ScPDGFNXA. Lane 5 contains a strong band at the expected molecular weight of around 70 kDa, which corresponds to unmodified 4070A envelope protein. All chimeric constructs were incorporated into retroviral particles at a slightly reduced efficiency relative to unmodified 4070A Env. PDGFNG4SA and PDGFNXA (lanes 1 and 2, respectively) were approximately 15 kDa larger than unmodified 4070A Env, due to the presence of the displayed PDGF-B domain. ScPDGFNG4SA and ScPDGFNXA (lanes 3 and 4, respectively) were approximately 30 kDa larger than 4070A Env, due to the display of two PDGF-B domains in a single chain per Env subunit. In all immunoblots a lower molecular weight non-specific band was observed of about 60 kDa. Moloney envelope constructs gave similar results to 4070A envelope constructs although incorporation levels were lower.

The aim of the work described in this disclosure was to generate single-chain forms of PDGF and VEGF displayed on retroviral envelopes that were capable of binding to their respective receptors. Single chain forms of these cytokines were constructed in an

attempt to display biologically active dimers as functional units, one per Env subunit (see Figure 3). To determine whether our constructs formed such dimers, it was necessary to show that no inter-molecular disulfide bonds were formed between proteins displayed on adjacent Env subunits. Single-domain forms of PDGF and VEGF were displayed as controls to demonstrate that such cross-links would occur. Non-reducing gels were used to test for the formation of inter-molecular disulfide bonds (see Figure 6). Lane 5 (4070A unmodified envelope protein) contained strong bands at approximately 70 kDa, which corresponded to SU, and 85 kDa, which corresponded to a small proportion of SU which had become disulfide bonded to TM. In contrast, the lanes 1 and 2, corresponding to PDGFNG4SA and PDGFNXA, contained only a faint band at 85 kDa, the expected size of the chimeric envelope proteins. A very strong band was observed at 170 kDa, double their size observed on standard reducing gels. This demonstrated that PDGF-B domains formed intermolecular disulfide bonds between adjacent envelope proteins, which caused them to run as disulfide linked dimers on the non-reducing gel. Lanes 3 and 4, corresponding to ScPDGFNG4SA and ScPDGFNXA, contained a strong band at about 100 kDa and a weaker band at about 15 kDa larger, a similar profile to that of the unmodified 4070A Env. Thus, these chimeric envelopes were not dimerizing with each other *via* disulfide bonds and therefore the displayed single chain dimers must have formed intra-molecular disulfide bonds.

Figure 7 shows an immunoblot of the same envelope proteins which had been treated (+) or had not been treated (-)with factor Xa protease prior to loading (+) or had not (-). Factor Xa protease treatment had no effect on any of the envelope proteins except for those containing factor Xa cleavage sites. Both PDGFNXA (2) and scPDGFNXA (5) produced the same band profile as unmodified 4070A Env, following factor Xa cleavage. This demonstrated that the displayed domains could be cleaved off by factor Xa protease, when a factor Xa site was present between the displayed domain and the underlying envelope protein. In addition, PDGFNXA must have been dimerizing *via* the displayed domain (PDGF) since its removal converted the remaining envelope proteins into monomers. Similar results were obtained for PDGFNG4SMo, PDGFNXMo, ScPDGFNG4SMo and ScPDGFNXMo.

Vectors bearing the chimeric envelope proteins were used to infect PDGF receptor-positive NIH 3T3 cells to show that the displayed single chain forms were capable of binding their corresponding receptors. Previously it had been shown that displayed ligands that are capable of binding to tyrosine kinase receptors impair viral entry by sequestering vector onto this non-permissive receptor, away from the virus receptor. Addition of agonistic free ligand negates the impairment. The observed infectious profile on ligand-receptor positive cells can therefore be used as a demonstration that a displayed ligand is binding to its receptor (Chadwick *et al.,* 1999). NIH 3T3 cells were infected with ScPDGFNG4SA vectors in the presence of increasing concentrations of PDGF-BB free ligand (see Figure 8). PDGF-BB effected an increase in viral titre at a concentration as low as 100 pM and gave optimal viral titre at 5 nM. No such effect was observed on the titre of vectors bearing unmodified 4070A envelope proteins (data not shown). These results demonstrate that displayed single chain PDGF can bind to PDGF receptors on NIH 3T3 cells.

EXAMPLE 3

VEGF Display

Eight vectors were generated that displayed VEGF-Env chimaeras. The chimeric envelope proteins consisted of a displayed domain (either single-domain VEGF or singlechain dimer VEGF (scVEGF)), which was linked *via* a short peptide, either AAAGGGS (SEQ ID NO. 8) or the factor Xa site AAAIEGR (SEQ ID NO:9), to the underlying envelope protein (4070A or Moloney).

Unmodified 4070A Env, Moloney Env, and the eight VEGF chimeric Env expression plasmids were transfected into Te1CeB6 packaging cell line by Superfect (Qiagen) according to the manufacturer's recommendations.

Iminunoblotting of pelleted virus was performed to determine whether the chimeric envelope proteins were incorporated into vector particles. Figure 9 shows an immunoblot of chimeric vectors VEGFNG4SA, VEGFNXA, scVEGFNG4SA and scVEGFNXA showing high levels of incorporation. Note the non-specific low molecular weight band, at approximately 60 kDa, common to all lanes. Chimeric Moloney envelope vectors gave

similar results to chimeric 4070A envelope vectors although a lower level of incorporation was visible.

To determine if disulfide bonds were forming between displayed VEGF subunits, the chimeric vectors were investigated using non-reducing gels (see Figure 10). Two bands were observed for unmodified 4070A Env, corresponding to SU (70 kDa) and SU-TM (85 kDa). In contrast, one band was observed at about 170 kDa for VEGFNG4SA and VEGFNXA, demonstrating that these envelope proteins associated as dimers, due to the intermolecular association of the displayed VEGF domains. Upon treatment with Factor Xa protease (+), the apparent mobility of the VEGFNG4SA dimer band remained unchanged. However, VEGFNXA dimer was reduced to the size of monomeric 4070A Env (lane 2). Thus, the dimerization was due to the association between displayed domains on adjacent envelope proteins. scVEGF.NG4SA and scVEGF.NXA envelope proteins produced bands at 100 kDa and 115 kDa, demonstrating that the displayed single chain proteins were capable of forming intra-molecular disulfide bonds between CKGF domains. Another band was observed at 200 kDa, demonstrating that some inter-molecular dimerization was still occurring between adjacent envelope proteins. Upon factor Xa treatment, the mobility of the scVEGFNG4SA bands remained unchanged. The band corresponding to dimeric scVEGFNXA disappeared, confirming that some of the displayed proteins had cross-linked adjacent Env subunits, and that this cross linking was removed when the displayed domain was cleaved off. The remaining bands corresponded to the size of unmodified 4070A Env (lane 9). Similar results were obtained for VEGF.NG4SMo, VEGF.NXMo, scVEGF.NG4SMo and scVEGF.NXMo.

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CLAIMS

1. A recombinant fusion protein comprising a first polypeptide and a second polypeptide fused to each other via peptide bonding, wherein the first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form a homo-oligomeric ligand which has the ability to bind to a receptor.

2. The recombinant fusion protein of claim 1 wherein the first polypeptide is a dimer or a trimer.

3. A recombinant fusion protein comprising a first polypeptide and a second polypeptide fused to each other via peptide bonding, wherein the first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form an oligomeric ligand, a said domain comprises a disulfide bonded growth factor, and wherein said oligomeric ligand has the ability to bind to a receptor.

4. The recombinant fusion protein of claim 1 or 3 wherein the first polypeptide further comprises a third domain which associates with the first and second domains to form said ligand.

5. The recombinant fusion protein of any one of the preceding claims, wherein the said ligand is a growth factor.

6. The recombinant fusion protein of claim 5 wherein the growth factor is a cysteine knot growth factor.

7. The recombinant fusion protein of claim 6 wherein the cysteine knot growth factor is a growth factor having a PDGF-like family signature.

8. The recombinant fusion protein of any one of the preceding claims, wherein the second polypeptide is anchored to a solid support.

9. The recombinant fusion protein of claim 8 wherein the solid support is a lipid enveloped particle or a biological membrane.

10. The recombinant fusion protein of any one of the preceding claims wherein the second polypeptide is a glycoprotein.

11. The recombinant fusion protein of claim 10 wherein the glycoprotein comprises a substantially intact envelope protein of a virus which enhances fusion between a lipid enveloped particle and a cellular membrane.

12. The recombinant fusion protein of claim 11 wherein the glycoprotein is a viral envelope protein.

13. The recombinant fusion protein of claim 11 wherein the virus is a retrovirus.

14. The recombinant fusion protein of claim 13 wherein the retrovirus is a mammalian type-C retrovirus.

15. The recombinant fusion protein of claim 14 wherein the mammalian type-C retrovirus is selected from the group consisting of a 4070A virus, a Moloney murine leukemia virus, and a gibbon ape leukemia virus.

16. The recombinant fusion protein of any one of the preceding claims, further comprising a protease recognition site between the first and second polypeptides.

17. The recombinant fusion protein of claim 1 further comprising an amino acid linker sequence between the first and second domains of the first polypeptide.

18. A recombinant fusion protein comprising a first polypeptide and a second polypeptide fused to each other via peptide bonding, wherein the first polypeptide comprises at least two subunits of a cysteine knot growth factor, wherein the second polypeptide comprises a substantially intact viral envelope protein which enhances fusion between a cellular membrane and a viral membrane, and wherein the at least two subunits of the cysteine knot growth factor associate to form a cysteine knot growth factor which binds to a cysteine knot growth factor receptor.

19. The recombinant fusion protein of claim 18, wherein the cysteine knot growth factor is a growth factor having a PDGF-like family signature.

20. The recombinant fusion protein of claim 18 or 19, wherein the viral envelope protein is a mammalian type-C retrovirus envelope protein.

21. The recombinant fusion protein of claim 20 wherein the mammalian type-C retrovirus envelope protein is selected from the group consisting of a 4070A envelope protein, a Moloney MLV envelope protein, and a gibbon ape leukemia virus envelope protein.

22. A lipid enveloped viral display package, comprising a recombinant fusion protein in accordance with any one of the preceding claims, wherein the second polypeptide of the fusion protein is anchored to the viral lipid envelope.

23. A library of viral display packages, comprising a plurality of viral display packages of claim 22.

24. A method of screening a test substance for the ability to modulate binding of an oligomeric ligand to a receptor, comprising the steps of:

contacting a lipid enveloped particle and a target cell with a test substance, wherein lipid enveloped particle comprises (a) a transferable label and (b) a recombinant fusion protein present on the outer surface of the lipid enveloped particle and comprising a first and a second polypeptide, wherein the first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form an oligomeric ligand, which has the ability to bind to a receptor present on the outer surface of the target cell; and

measuring the amount of the transferable label in the target cell in the presence of the test substance, wherein an increase in the amount of transferable label in the target cell relative to the amount of transferable label in a target cell in the absence of the test substance indicates reduction of binding of the oligomeric ligand to the receptor.

25. The method of claim 24, wherein the lipid enveloped particle is a virus.

26. The method of claim 24 or 25, wherein the virus is a retrovirus.

27. The method of any one of claims 24, 25 or 26, wherein the second polypeptide enhances fusion between the particle and the target cell.

28. The method of any one of claims 24-27, wherein the second polypeptide is of viral origin.

29. The method of any one of claims 24-28, wherein the second polypeptide is a substantially intact viral envelope protein.

30. The method of any one of claims 24-29, wherein the second polypeptide is a mammalian type-C retrovirus envelope protein.

31. The method of claim 30 wherein the mammalian type-C retrovirus envelope protein is selected from the group consisting of a 4070A envelope protein, a Moloney MLV envelope protein, and a gibbon ape leukemia virus envelope protein.

32. The method of any one of claims 24-31, wherein the target cell is a eukaryotic cell.

33. The method of claim 32, wherein the target cell is a mammalian cell.

34. The method of claim 32 or 33, wherein the target cell is a human cell.

35. The method of any one of claims 24-34, wherein the transferable label is a reporter gene which encodes a detectable product.

36. A method of targeting a transferable label to a target cell, comprising the step of:

contacting a target cell with a lipid enveloped particle, wherein the lipid enveloped particle comprises (a) a transferable label and (b) a recombinant fusion protein present on the outer surface of the lipid enveloped particle and comprising a first and a second polypeptide, wherein the first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form an oligomeric ligand which has

the ability to bind to a receptor present on the outer surface of the target cell, whereby a lipid enveloped particle-target cell complex is formed.

37. A method of delivering a transferable label to a target cell, comprising the steps of:

contacting a target cell with a lipid enveloped particle, wherein the lipid enveloped particle comprises

(a) a transferable label; and

(b) a recombinant fusion protein present on the outer surface of the lipid enveloped particle and comprising a first and a second polypeptide separated by a protease recognition site, wherein the first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form an oligomeric ligand which has the ability to bind to a receptor present on the outer surface of the target cell, whereby a lipid enveloped particle-target cell complex is formed;

and contacting the lipid enveloped particle-target cell complex with a protease which recognizes the protease recognition site, whereby the first polypeptide is cleaved from the second polypeptide and the transferable label is transferred to the target cell.

38. The method of claim 36 or 37 wherein the target cell is a eukaryotic cell.

39. The method of claim 36 or 37 wherein the eukaryotic cell is a mammalian cell.

40. The method of claim 36 or 37 wherein the mammalian cell is a human cell.

41. The method of claim 36 or 37 wherein the target cell is *in vitro*.

42. The method of claim 36 or 37 wherein the target cell is in a mammalian body.

43. A method of screening a test substance for the ability to dissociate an oligomeric ligand into its constituent subunits, comprising the steps of:

contacting a target cell with a viral particle, wherein the viral particle comprises (a) a transferable label and (b) a recombinant fusion protein present on the outer surface of the viral particle and comprising a first and a second polypeptide, wherein the first polypeptide comprises first and second domains in a continuous polypeptide chain which self-associate to form an oligomeric ligand which has the ability to bind to a receptor present on the outer surface of the target cell; and measuring the amount of transferable label in the target cell in the presence of the test substance, wherein an increase in the amount of transferable label in the target cell relative to the amount of transferable label in a target cell in the absence of the test substance indicates dissociation of an oligomeric ligand into its constituent subunits.

44. An isolated polynucleotide which encodes a recombinant fusion protein comprising a first polypeptide and a second polypeptide fused to each other via peptide bonding, wherein the first polypeptide comprises at least two subunits of a cysteine knot growth factor with a characteristic PDGF-like family signature selected from the group consisting of PDGF and VEGF, wherein the second polypeptide comprises a substantially intact viral envelope protein selected from the group consisting of a 4070A envelope protein, a Moloney MLV envelope protein, and a gibbon ape leukemia virus protein.

Fig.1.

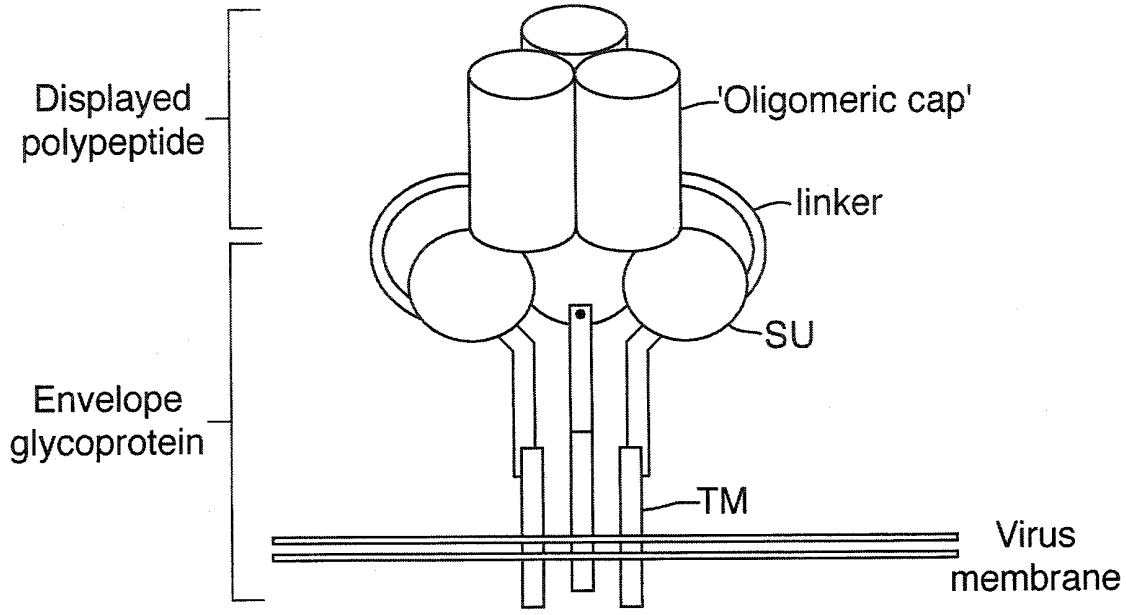

Displayed polypeptide
Envelope glycoprotein
'Oligomeric cap'
linker
SU
TM
Virus membrane

Fig.2.

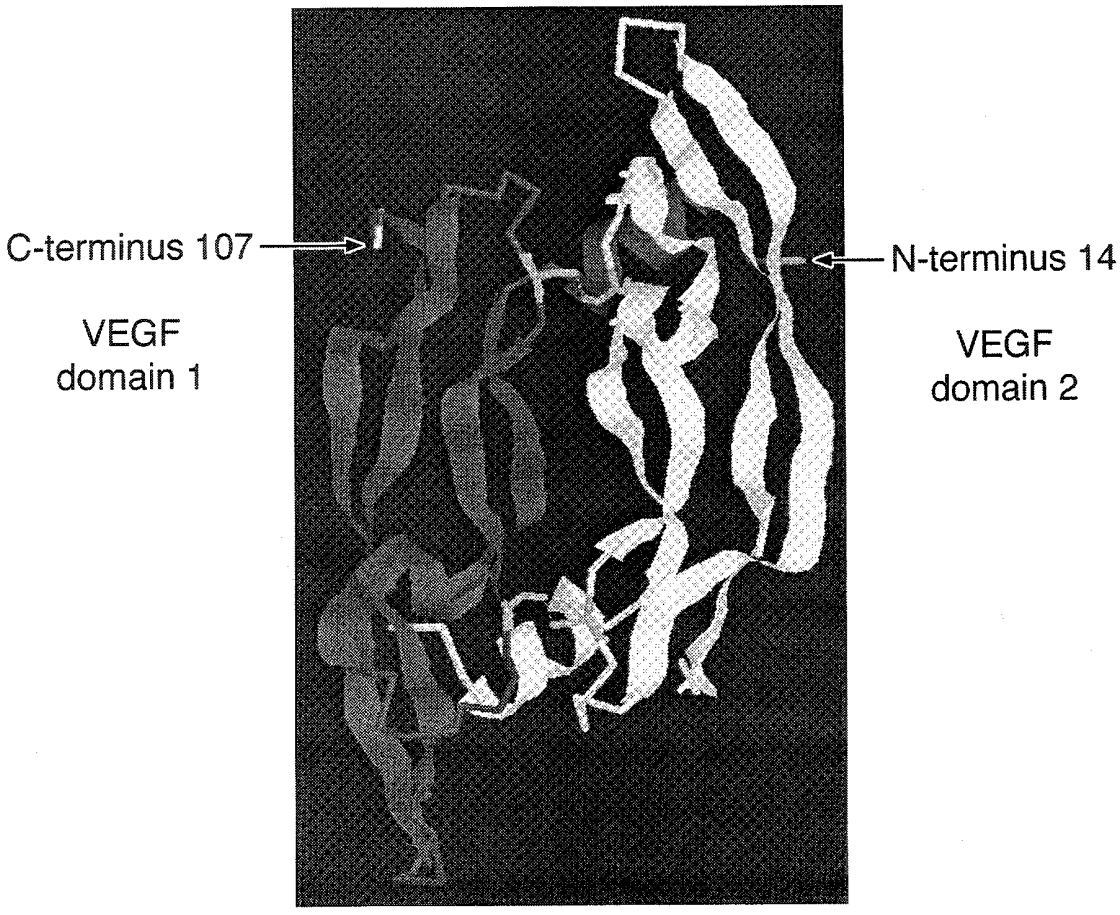
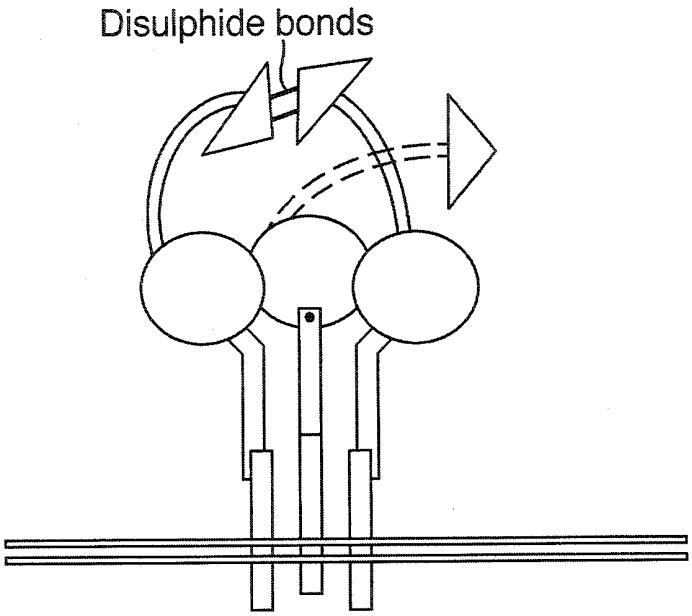
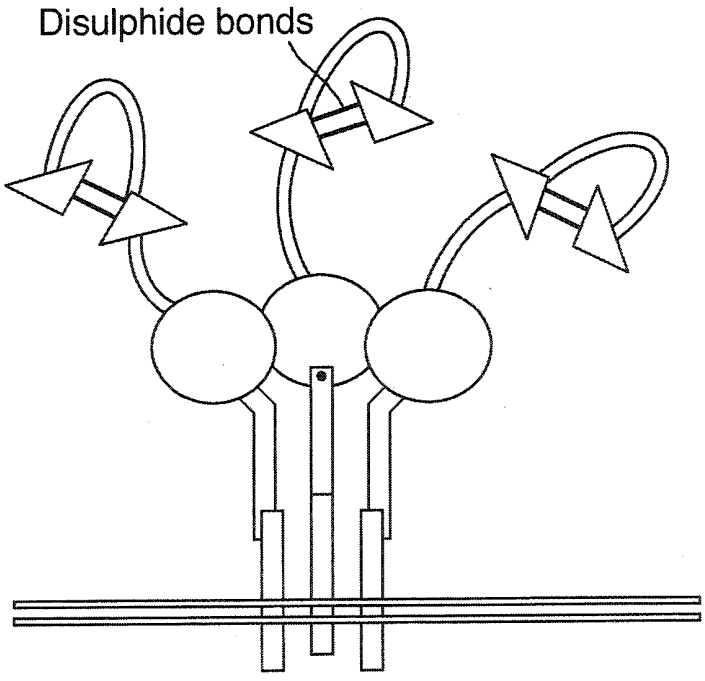

C-terminus 107
VEGF domain 1
N-terminus 14
VEGF domain 2

Fig.3(a).



CKGF domain display

Fig.3(b).



scCKGF dimer display

Fig.4(a).

Displayed polypeptide
linker
Envelope protein (SU+TM)
PDGF-B
VEGF
G4S
Xa site
4070A Env
Moloney Env
Nomenclature:
PDGFN
VEGFN
G4S
X
A
Mo

Fig.4(b).

Displayed polypeptide
hinge
Displayed polypeptide
linker
Envelope protein (SU+TM)
PDGF-B
G4S
PDGF-B
VEGF
G4S
VEGF
G4S
Xa site
4070A Env
Moloney Env
Nomenclature:
scPDGFN
scVEGFN
G4S
X
A
Mo

Fig.5.

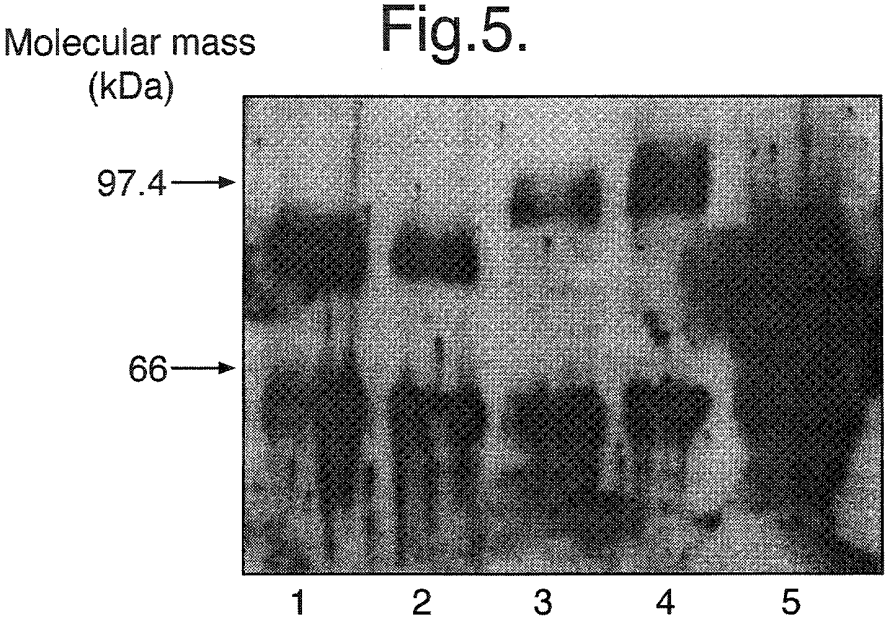

Molecular mass
(kDa)
97.4
66
1
2
3
4
5

Fig.6.

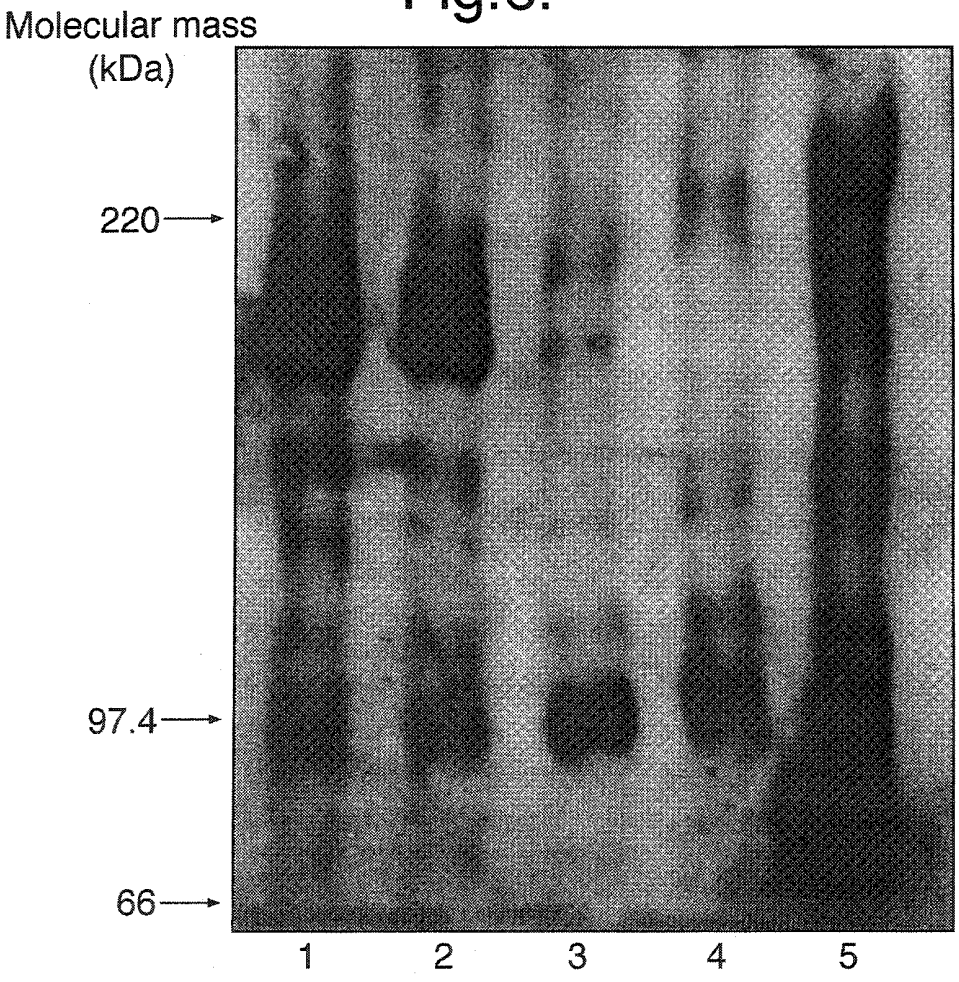

Molecular mass
(kDa)
220
97.4
66
1
2
3
4
5

Fig.7.

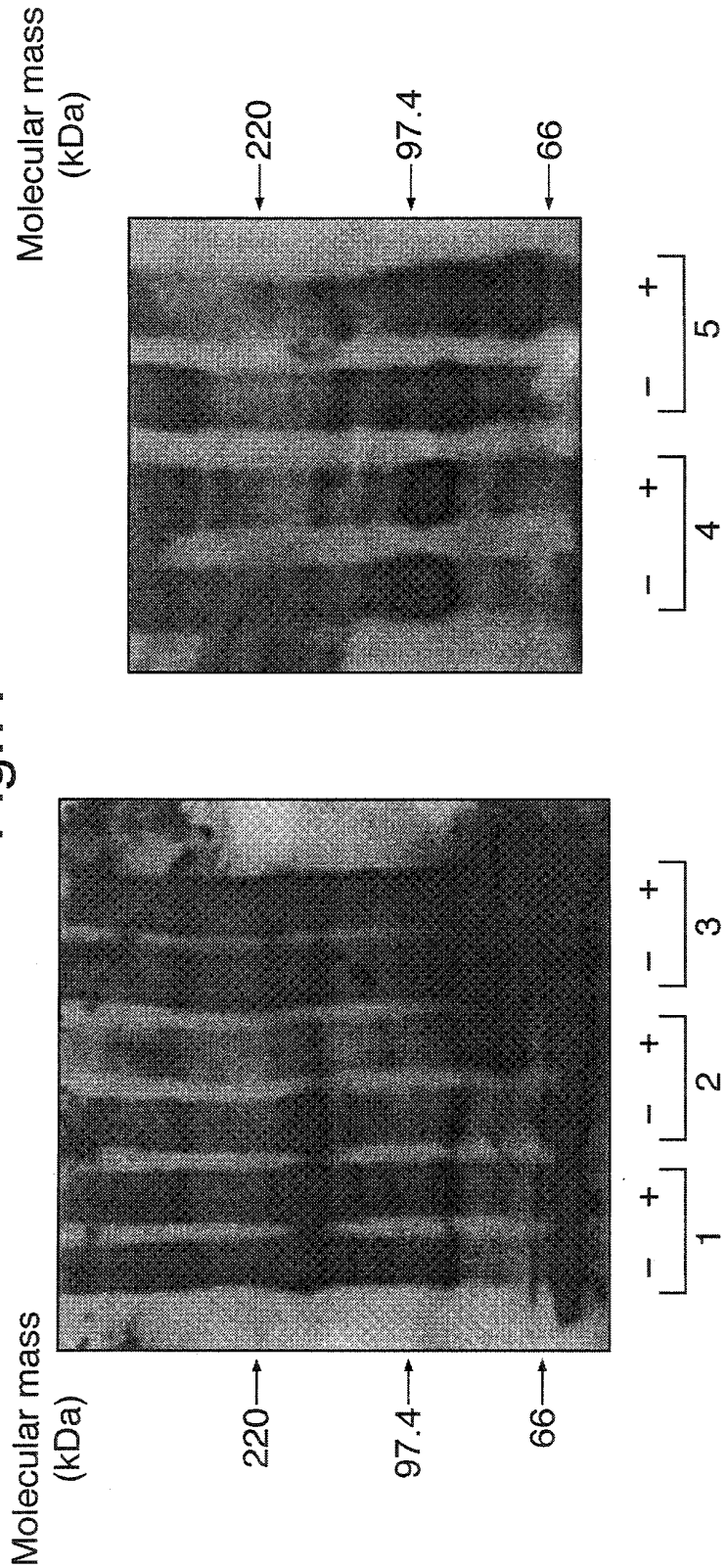


Fig.8.

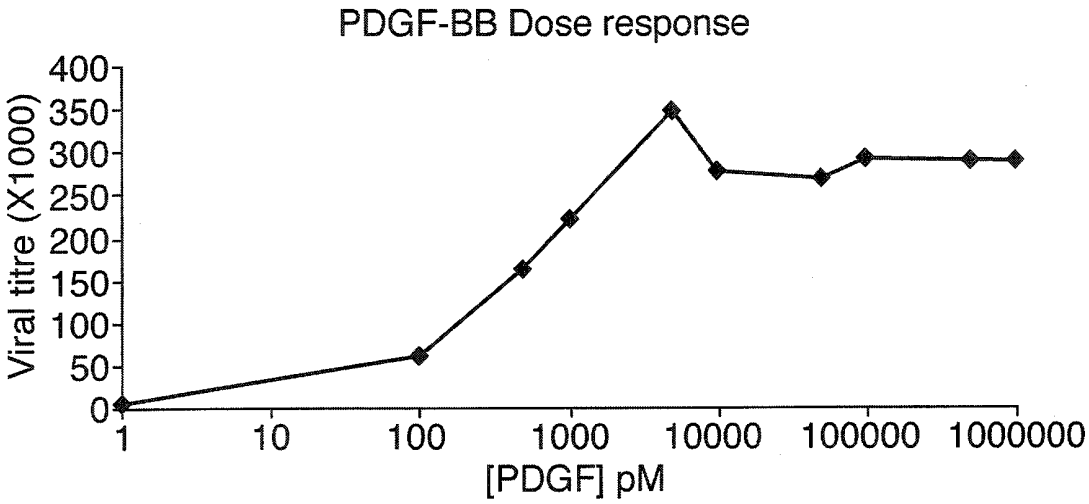

PDGF-BB Dose response
Viral titre (X1000)
400
350
300
250
200
150
100
50
0
1
10
100
1000
10000
100000
1000000
[PDGF] pM

Fig.9.

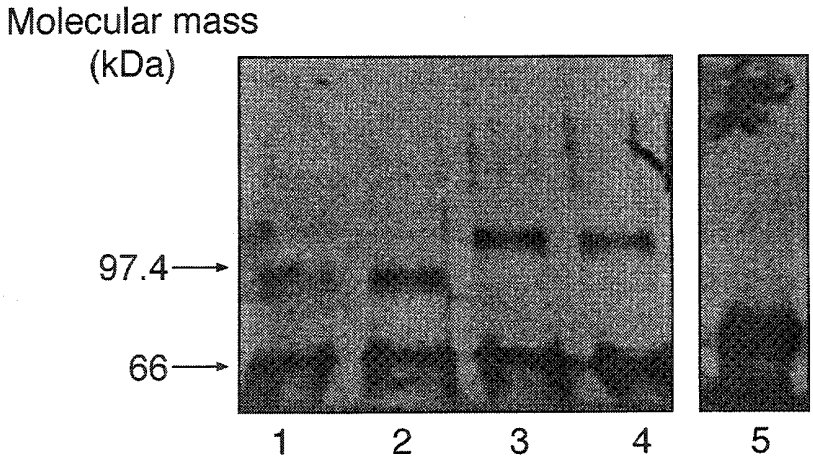
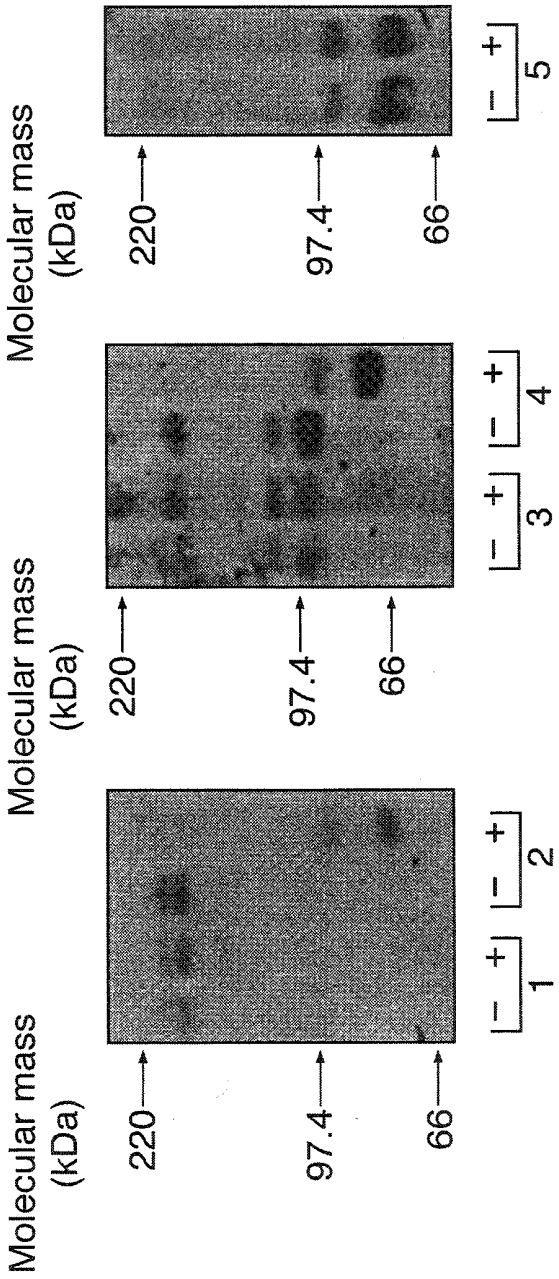

Molecular mass
(kDa)
97.4
66
1
2
3
4
5

Fig.10.


Molecular mass (kDa)
220
97.4
66
- +
1
- +
2
Molecular mass (kDa)
220
97.4
66
- +
3
- +
4
Molecular mass (kDa)
220
97.4
66
- +
5

SEQUENCE LISTING

<110> Chadwick, Mark P.
Russell, Stephen J.
Belcher, Craig
Glenn, Daphne
Bullough, Fran

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1 5

<210> 8

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<213> Homo sapiens

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1 5

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Ala Ala Ala Ile Glu Gly Arg

1 5

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<211> 4

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Ala Ala Pro Val

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Ala Ala Pro Val

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Ala Ala Pro Met

1

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Ala Ala Pro Phe

1

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Ala Ala Pro Lys

1

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<400> 15

Pro Pro Ser Cys Val Xaa Xaa Xaa Arg Cys Gly Ser Thr Ala Gly Cys

1 5 10 15

Cys