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(54) **Title**: FGFR-FC FUSION PROTEIN AND USE THEREOF

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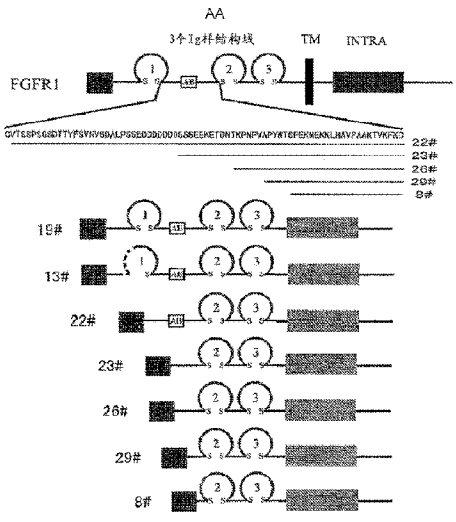

图 1 / Fig. 1

AA 3 IG-LIKE DOMAINS

(57) **Abstract**: Provided are a fusion protein comprising the intermediate functional sequence (IFS) part of the FGFR Ig-like domain, FGFR second Ig-like domain (D2), FGFR third Ig-like domain (D3), and Immunoglobulin Fc region, nucleic acid molecule encoding the fusion protein, and the carrier and host cell comprising the nucleic acid molecule. Also provided are a preparation method for the fusion protein and the use of carriers or cells comprising the fusion protein, the nucleic acid molecule encoding the fusion protein, or the nucleic acid molecule in preparing medications for treating diseases related to angiogenesis regulation.

(57) **摘要**: 提供了一种包含源自 FGFR Ig 样结构域中间功能性序列 (IFS) 的部分、FGFR 第二 Ig 样结构域 (D2) 和 FGFR 第三 Ig 样结构域 (D3) 和免疫球蛋白 Fc 区的融合蛋白、编码该融合蛋白的核酸分子以及包含核酸分子的载体和宿主细胞。还提供了该融合蛋白的制备方法，以及包含融合蛋白、编码融合蛋白的核酸分子或包含核酸分子的载体或细胞在制备用于治疗与血管新生调控相关的疾病的药物中的应用。

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根据细则 4.17 的声明:

— 关于发明人身份(细则 4.17(i))

— 关于申请人有权申请并被授予专利(细则 4.17(ii))

本国际公布:

— 包括国际检索报告(条约第 21 条(3))。

— 包括说明书序列表部分(细则 5.2(a))。

FGFR-Fc FUSION PROTEIN AND USE THEREOF

FIELD OF INVENTION

The present invention belongs to the field of biotechnology and relates to the treatment of diseases, especially the treatment of FGF overexpression-related diseases. Particularly, the present invention relates to FGFR-Fc fusion proteins and the use thereof in the treatment of angiogenesis regulation-related diseases. More particularly, the present invention relates to isolated soluble FGFR-Fc fusion proteins and their applications in manufacture of the medicament for the treatment of angiogenesis regulation-related diseases.

BACKGROUND OF THE INVENTION

Angiogenesis is one of the primary factors resulting in the growth and metastasis of malignant tumor [1]. The process of angiogenesis is regulated by many factors, among which some factors promote angiogenesis, while some factors inhibit angiogenesis, and as a result, the regulation of angiogenesis is a very complicated dynamic equilibrium process [2]. The anti-angiogenesis treatment is intended to control the growth of tumor by blocking angiogenic stimulating factors or preventing angiogenesis in the tumor using angiogenesis inhibitors. At present, a large amount of angiogenic stimulating factors are known, such as, for example, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF) etc., which may stimulate the division and differentiation of vascular endothelial cells and the morphogenesis of blood vessels. Among these factors mentioned above, it is now known that VEGF is the most angiogenesis-specific and the most effective growth factor [3, 4].

In a hypoxic environment inside the tumor tissue, a plenty of VEGFs are secreted by the tumor cells, which induce the division and migration of vascular

endotheliocytes, finally resulting in the establishment of tumor vascular network. It has been demonstrated by a lot of animal experiments that the inhibition of VEGF may prevent angiogenesis, and further inhibit the growth of tumor. For this reason, VEGF and its receptors become important targets for anti-angiogenesis medicaments. At present, anti-angiogenesis medicaments demonstrated in clinical trials to have remarkable efficacy include Bevacizumab (under the trade name of Avastin), which is able to block VEGF directly and inhibit the tumor angiogenesis. Bevacizumab was approved for marketing by FDA in 2004, and as a first-line drug for rectal cancer, it is the first marketing-approved drug that plays a role in anticarcinogenesis by inhibiting angiogenesis. Avastin is a humanized anti-VEGF monoclonal antibody, which is produced by a famous US biotechnology company Genentech. In a large-scale Phase III clinical trial, the combined therapy by Avastin and chemotherapy may significantly extend the survival time of the patients suffered from many kinds of cancers, including rectal cancer, lung cancer, breast cancer and renal cancer, etc. [5, 6] The clinical success of Avastin is a landmark, demonstrating that the anti-angiogenesis treatment using tumor vascular system as the target is a clinically effective measure and provide a new path for the tumor treatment. In western countries, Avastin has already been widely used for tumor therapy and is one of the drugs holding the largest global market share.

Besides Avastin, several drugs for anti-VEGF signaling are also under the late phase of human clinical trial in foreign countries, which are expected for clinical application in the next several years. Among others, Aflibercept (also called as VEGF-Trap), developed by the cooperation between US biotechnology company Regeneron and Sanofi-Aventis, is now under the large-scale Phase III clinical trial [7]. An anti-VEGF receptor II (VEGFR2) monoclonal antibody drug IMC-1121B (Imclone) is also under the Phase III clinical trial [8]. In China, the development of new medicaments now also focuses on the anti-tumor medicament using anti-angiogenesis as the target. Medicaments using VEGF and its receptor, or other angiogenic targets are under the development by several Chinese companies or

research institutions. These new drugs will definitely provide new choices for cancer therapy and new hope for the patients.

Great progress has been achieved in the clinical treatment of tumor using anti-VEGF medicament, however, it has also been shown by the clinical trial that the anti-VEGF treatment are also considerably limited. From the point of the effect of tumor treatment, Avastin may extend the half survival time of the colon cancer patient for about 3-4 months [9, 10], and extend the half survival time of the breast cancer patient for about 7-8 months [11], and thus, Avastin cannot effectively inhibit the growth of tumor blood vessel in a long term. Therefore, the problem how to further improve the effect of clinical treatment using anti-angiogenesis method need to be solved by tumor investigators and is also the main point of the research and development of the next generation anti-angiogenesis medicament.

The primary causes resulting in the failure of anti-VEGF treatment or the appearance of resistance may depend on the regulation of tumor angiogenesis by a plurality of factors. Although VEGF plays an important role in angiogenesis, it is not the only angiogenesis stimulating factor. Meanwhile, owing to the heterogeneity of tumor cells, the complexity of tumor microenvironment and the compensatory response mechanism of body, when the activity of VEGF is inhibited for a long period of time, other angiogenesis stimulating factors would be expressed [12], and thus the growth of tumor blood vessel is no longer dependent on VEGF signaling path. The variation of angiogenesis factors expressed by the tumor was studied during anti-VEGFR2 treatment for pancreatic tumor by Prof. Hanahan's group (University of California, San Francisco, US), indicating that the expression of several genes changed during anti-VEGF treatment, in which the expression of FGF-2 significantly increased. It has been shown that the expression of FGF, especially FGF-2, increased significantly in the tumor resistant to anti-VEGF treatment so that angiogenesis was activated again and the tumor repopulation was inhibited after blocking FGF signal pathway [13]. It may be seen that the over-expression of FGF-2 is closely related to the ability of tumor to escape from anti-VEGF treatment. Therefore, we believe the

angiogenesis of tumor may be efficiently prevented and the tumor growth may be inhibited by blocking FGF pathway, so that angiogenesis-related diseases can be treated alone by anti-FGF treatment or by a combination therapy of anti-FGF and anti-VEGF treatment.

Fibroblast growth factor (FGF) is a growth factor family for heparin-binding, and there are 22 family members (FGF 1-14, 16-23) in the mammals. FGF plays an important role in many biological functions, for example, cell proliferation, differentiation, migration, angiogenesis and tumorigenesis etc. Fibroblast growth factor receptor (FGFR) is the receptor which binds the family members of fibroblast growth factor. FGF may bind FGFR and activate the downstream signal pathway, which plays an important role in a physiological and pathological process, such as embryogenesis, development, vasculogenesis, vasodilatation, neuroregulation, ischemia protection, wound healing and tumorigenesis etc. [14, 15]It has already been demonstrated that overexpression of FGF/FGFR *in vivo* is closely related to many diseases including tumors (such as fibroma, neuroglioma, melanoma, prostate carcinoma, lymphomata, leukaemia, urinary system cancer etc.), skeletal system diseases (dwarfism, craniosynostosis, achondroplasia, acanthosis nigricans) and renal failure etc. It has already been reported that increased expression level of FGF and its receptor may directly promote the survival and proliferation of tumor cells, and the survival of hepatic carcinoma cells is significantly reduced by down-regulation of FGF by siRNA [22]. Therefore, it is expected to block FGF pathway by constructing an FGFR-Fc fusion protein that is able to antagonize FGF, which is of the potential for treating FGF overexpression-related diseases.

At present, few researches focus on the development of new anti-angiogenesis medicament using FGF and its receptor as the target in clinical trials. For example, FP-1039, a fusion protein composed of whole extracellular domain of human FGFR1 and human IgG1 Fc fragment, is developed by a US company Five Prime and now in volunteer recruitment stage of Phase I clinical trail. However, it has been suggested by researches of Wang and Olsen that the first Ig-like domain of the

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extracellular domain of human FGFR1 and the linking fragment between the first and the second Ig-like domain of the extracellular domain of human FGFR1 may inhibit binding of FGFR1 and FGF [20, 21].

Therefore, it is expected to block FGF pathway by constructing an FGFR-Fc fusion protein that is able to antagonize FGF so that angiogenesis may be efficiently inhibited or it may act on tumor cells directly and inhibit their growth, and it is of the potential for treating FGF overexpression-related diseases to cure angiogenesis-related diseases such as tumors.

It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

SUMMARY

The space structure of protein is closely related to its biological function. The FGF binding capacity is directly influenced by difference among the conformations of each Ig-like domain of the extracellular domain of FGFR and the linking fragment. Different fusion proteins, composed of the FGFR extracellular domain fragments of various lengths and IgG Fc, are constructed by means of genetic engineering to obtain fusion proteins with different conformations, so that the fusion protein with high efficiency of FGF binding and biological activity can be screened.

There are four FGFR genes in the mammals: fgfR1-fgfR4. Fibroblast growth factor receptor is composed of the extracellular domain, transmembrane domain and intracellular domain. There are many members in FGFR family, which have similar extracellular domain but vary in the ligand binding property and kinase domain. Their extracellular domains include three immunoglobulin-like (Ig-like) domains: the first Ig-like domain, the second Ig-like domain and the third Ig-like domain, and there is a sequence between the first and the second Ig-like domain, which is referred as the intermediate functional sequence of the Ig-like domain of FGFR (IFS for short herein)

in this specification. The intermediate functional sequence may comprise one acidic amino acid segment, which is referred as acidic box (AB).

In a first aspect, the invention provides an isolated soluble fusion protein of fibroblast growth factor receptor (FGFR), comprising: a part derived from the FGFR extracellular domain and an immunoglobulin Fc region, wherein the part derived from the FGFR extracellular domain consists of an amino acid sequence corresponding to positions 1-241 of SEQ ID NO: 12, positions 1-230 of SEQ ID NO: 13, or positions 1-224 of SEQ ID NO: 14 or encoded by a nucleotide sequence corresponding to positions 1-723 of SEQ ID NO: 19, positions 1-690 of SEQ ID NO: 20, or positions 1-672 of SEQ ID NO: 21.

In a second aspect, the invention provides an isolated soluble fusion protein of fibroblast growth factor receptor (FGFR), wherein said protein consists of an amino acid sequence of any one of SEQ ID NOs: 12-14, or is encoded by a nucleic acid molecule consisting of a nucleotide sequence of any one of SEQ ID NOs: 19-21.

Disclosed herein is an isolated soluble fusion protein of fibroblast growth factor receptor (FGFR), which comprises: the part derived from the intermediate functional sequence (also referred as IFS herein) of the Ig-like domain of FGFR, the second Ig-like domain (also referred as D2 herein) of FGFR, the third Ig-like domain (also referred as D3 herein) of FGFR and immunoglobulin Fc region.

Also disclosed is a fusion protein, which comprises or consists of: the part derived from the intermediate functional sequence region of the Ig-like domain of FGFR, the second Ig-like domain of FGFR, the third Ig-like domain of FGFR and immunoglobulin Fc region. In some embodiments, the part derived from IFS contains no acidic box. In some other embodiments, the part of IFS has the amino acid sequence of position 134 to position 162, position 145 to position 162 or position 151 to position 162 of SEQ ID NO: 1, or has the amino acid sequence sharing at least 90% identity, preferably 93%, 95%, 97%, 98% or 99% identity, with the amino acid sequence of position 134 to position 162, position 145 to position 162 or position 151 to position 162 of SEQ ID NO: 1.

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Also disclosed is a fusion protein, which comprises or consists of: the first Ig-like domain (also referred to as D1 herein) of FGFR or a moiety thereof, the part derived from the intermediate functional sequence region of the Ig-like domain of FGFR, the second Ig-like domain of FGFR, the third Ig-like domain of FGFR and immunoglobulin Fc region. Preferably, said D1 domain or a moiety thereof possesses:

the amino acid sequence corresponding to position 40 to position 118 of SEQ ID NO: 1, or the amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with the sequence of position 40 to position 118 of SEQ ID NO: 1; or

the amino acid sequence corresponding to position 77 to position 118 of SEQ ID NO: 1, or the amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with the amino acid sequence of position 77 to position 118 of SEQ ID NO:1.

Also disclosed is a fusion protein, which comprises or consists of: the intermediate functional sequence region of the Ig-like domain of FGFR or a moiety thereof, the second Ig-like domain of FGFR, the third Ig-like domain of FGFR and immunoglobulin Fc region, wherein:

the second Ig-like domain of FGFR has the amino acid sequence corresponding to position 163 to position 247 of SEQ ID NO: 1, or the amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with the amino acid sequence of position 163 to position 247 of SEQ ID NO: 1; and/or

the third Ig-like domain of FGFR has the amino acid sequence corresponding to position 270 to position 359 of SEQ ID NO: 1, or the amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with the amino acid sequence of position 270 to position 359 of SEQ ID NO: 1.

Also disclosed is a fusion protein, which comprises a region derived from the extracellular domain of FGFR and immunoglobulin Fc region or composed thereof, wherein the region derived from the extracellular domain of FGFR:

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(1) comprises an amino acid sequence corresponding to any one of positions 1-353 of SEQ ID NO: 9, positions 1-299 of SEQ ID NO: 10, positions 1-273 of SEQ ID NO: 11, positions 1-241 of SEQ ID NO: 12, positions 1-230 of SEQ ID NO: 13, positions 1-224 of SEQ ID NO: 14 and positions 1-219 of SEQ ID NO: 15, or encoded by a nucleotide sequence corresponding to any one of positions 1-1059 of SEQ ID NO: 16, positions 1-897 of SEQ ID NO: 17, positions 1-819 of SEQ ID NO: 18, positions 1-723 of SEQ ID NO: 19, positions 1-690 of SEQ ID NO: 20, positions 1-672 of SEQ ID NO: 21, and positions 1-657 of SEQ ID NO: 22;

(2) comprises or consists of an amino acid sequence having at least 70% identity, optionally at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with an amino acid sequence corresponding to any one of positions 1-353 of SEQ ID NO: 9, positions 1-299 of SEQ ID NO: 10, positions 1-273 of SEQ ID NO: 11, positions 1-241 of SEQ ID NO: 12, positions 1-230 of SEQ ID NO: 13, positions 1-224 of SEQ ID NO: 14 and positions 1-219 of SEQ ID NO: 15; or

(3) comprises or consists of an amino acid sequence encoded by a nucleotide sequence having at least 70% identity, optionally at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with a nucleotide sequence corresponding to any one of positions 1-1059 of SEQ ID NO: 16, positions 1-879 of SEQ ID NO: 17, positions 1-819 of SEQ ID NO: 18, positions 1-723 of SEQ ID NO: 19, positions 1-690 of SEQ ID NO: 20, positions 1-672 of SEQ ID NO: 21 and positions 1-657 of SEQ ID NO: 22.

Also disclosed is a fusion protein, said protein:

(1) comprises the amino acid sequence indicated by any one of SEQ ID NOs: 9-15, or the amino acid sequence encoded by the nucleotide sequence indicated by any one of SEQ ID NOs: 16-22;

(2) comprises or consists of the amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity, with the amino acid sequence indicated by any one of SEQ ID NOs: 9-15; or

(3) comprises or consists of the amino acid sequence encoded by the nucleotide sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity, with the nucleotide sequence indicated by any one of SEQ ID NOs: 16-22.

Preferably, in the fusion protein, the immunoglobulin Fc region is human IgG1 Fc region, and more preferably, it comprises:

the amino acid sequence corresponding to SEQ ID NO: 7, or the amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity, with the amino acid sequence of SEQ ID NO: 7; or

the amino acid sequence encoded by the nucleotide sequence corresponding to SEQ ID NO: 8, or the amino acid sequence encoded by the nucleotide sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity, with the nucleotide sequence of SEQ ID NO: 8.

In one embodiment of the present invention, the immunoglobulin Fc region is located at the C-terminus of the fusion protein.

Also disclosed is a fusion protein precursor comprising a secretory signal peptide region, for example, VEGFR1 signal peptide region, and preferably, the secretory signal peptide region has the amino acid sequence of position 1 to position 26 of SEQ ID NO: 2 or the amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 23. Preferably, the signal peptide region is located at the N-terminus of the precursor.

Also disclosed is a fusion protein which sequentially comprises from the N-terminus to the C-terminus: the part derived from IFR, D2, D3 and immunoglobulin Fc region.

In another embodiment, the domains and/or regions involved in the fusion protein of the present invention are linked directly and/or by a linker. In one embodiment, the region derived from the extracellular domain of FGFR and immunoglobulin Fc region are linked directly. In another embodiment, the region derived from the extracellular domain of FGFR and immunoglobulin Fc region are linked by a linker.

In one embodiment, the fusion protein of the present invention inhibits angiogenesis. In another embodiment, the fusion protein of the present invention binds FGF, preferably FGF2, *in vivo* and/or *in vitro*. In another embodiment, the fusion protein of the present invention inhibits tumor cells directly.

Also disclosed is an FGFR-Fc fusion protein, which comprises a part derived from the extracellular domain of FGFR and a part derived from immunoglobulin Fc region. Particularly, the part derived from the extracellular domain of FGFR is the part derived from the extracellular domain of FGFR1. Preferably, the immunoglobulin Fc region is human immunoglobulin Fc region, for example, human IgG1 Fc region. In one embodiment of the present invention, the FGFR-Fc fusion protein has the capacity of binding and/or antagonizing FGF, and thus, may inhibit angiogenesis.

In the FGFR-Fc fusion protein, the part derived from the extracellular domain of FGFR may comprise one or more selected from the group consisting of: D1 domain or a moiety thereof, the part derived from IFS, D2 domain or a moiety thereof and D3 domain or a moiety thereof. In one embodiment, the part derived from the extracellular domain of FGFR may comprise D1 or a moiety thereof, the part derived from IFS, D2 domain and D3 domain. In another embodiment, the part derived from the extracellular domain of FGFR may comprise the part derived from IFS, D2 domain and D3 domain, and preferably, the part derived from IFS has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162 or position 151 to position 162 of SEQ ID NO: 1. In some preferable embodiments of the present invention, the FGFR-Fc fusion protein contains no D1 or a moiety thereof. In some other preferable embodiments of the present invention, the FGFR-Fc fusion protein contains no part from IFS other than the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162 or position 151 to position 162 of SEQ ID NO: 1.

In some embodiments of the present invention, the order from the N-terminus to the C-terminus of each region and/or each domain involved in the FGFR-Fc fusion protein may

be any order. In some other embodiments, said order can be as shown in Fig. 1. In some other embodiments, said order may be different from the order shown in Fig. 1.

In some embodiments, the FGFR-Fc fusion protein of the present invention further comprises one or more intrachain disulfide bonds, and preferably, comprises one or more intra-chain disulfide bonds in the Ig-like domain.

In one embodiment of the present invention, the FGFR-Fc fusion protein can be produced by expression of the nucleic acid comprising the nucleotide sequence indicated by any one of SEQ ID NOs: 16-22 in a mammalian cell line. Particularly, the mammalian cell line is CHO cell line.

Additionally, a FGFR-Fc fusion protein is also disclosed in which domains and/or regions involved in the fusion protein are operatively linked and/or by a linker.

In a third aspect, the invention provides an isolated nucleic acid molecule encoding the fusion protein of the first or second aspect.

Also disclosed is an isolated nucleic acid molecule which encodes the fusion protein or the precursor of the fusion protein of the present invention is provided. Preferably, the nucleic acid molecule comprises the nucleotide sequence indicated by any one of SEQ ID NOs: 16-22.

In a fourth aspect of the present invention, a vector comprising the nucleic acid molecule of the third aspect is provided.

In a fifth aspect of the present invention, an isolated and/or non-human cell, optionally a CHO cell, transfected by the vector of the fourth aspect provided.

Also disclosed is a composition comprising the fusion protein of the present invention, which is mixed with a pharmaceutically acceptable carrier, is provided.

In a sixth aspect of the present invention, a pharmaceutical composition comprising the fusion protein of the first or second aspect, the nucleic acid molecule of the third aspect, the vector of the fourth aspect, or the cell of the fifth aspect and a pharmaceutically acceptable carrier is provided.

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Also disclosed is a method for producing the angiogenesis-inhibitory fusion protein, which is carried out by expressing the fusion protein in prokaryotic cells or eukaryotic cells, especially, in mammalian cell lines.

Also disclosed is a method for producing the angiogenesis-inhibitory fusion protein, which is carried out by expressing the nucleic acid molecule in mammalian cell lines. Preferably, the mammalian cell line is CHO cell line.

In a seventh aspect, the invention provides use of the fusion protein of the first or second aspect, the nucleic acid molecule of the third aspect, the vector of the fourth aspect, or the cell of the fifth aspect in the manufacture of a medicament for inhibiting angiogenesis in a mammal.

In an eighth aspect, the invention provides use of the fusion protein of the first or second aspect, the nucleic acid molecule of the third aspect, the vector of the fourth aspect, or the cell of the fifth aspect in the manufacture of a medicament for treating or preventing a tumor, optionally a solid tumor, in a subject.

In a ninth aspect, the invention provides a method for inhibiting angiogenesis in a mammal in need thereof, comprising administering an effective amount of the fusion protein of the first or second aspect, the nucleic acid molecule of the third aspect, the vector of the fourth aspect, the cell of the fifth aspect, or the pharmaceutical composition of the sixth aspect to the mammal.

In a tenth aspect, the invention provides a method for treating or preventing a tumor, optionally a solid tumor, in a subject, comprising administering an effective amount of the fusion protein of the first or second aspect, the nucleic acid molecule of the third aspect, the vector of the fourth aspect, the cell of the fifth aspect, or the pharmaceutical composition of the sixth aspect to the subject.

Also disclosed is a method for inhibition of angiogenesis is provided, which comprises administrating angiogenesis-inhibiting effective amount of the FGFR-Fc fusion protein, the nucleic acid molecule encoding the protein, the vector comprising the nucleic acid molecule

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and/or a pharmaceutical composition comprising any one mentioned above to the subject in need thereof. Preferably, the method is carried out in the mammals.

Also disclosed is a method for the treatment or prevention of tumors in the mammals is provided, which comprises administrating therapeutically or preventively effective amount of the FGFR-Fc fusion protein, the nucleic acid molecule encoding the protein, the vector comprising the nucleic acid molecule and/or a pharmaceutical composition comprising any one mentioned above to the subject in need thereof, and preferably, the tumor is a solid tumor.

Also disclosed is a method for the treatment or prevention of ophthalmic angiogenesis-related diseases in the mammals is provided, which comprises administrating therapeutically or preventively effective amount of the FGFR-Fc fusion protein, the nucleic acid molecule encoding the protein, the vector comprising the nucleic acid molecule and/or a pharmaceutical composition comprising any one mentioned above to the subject in need thereof, and preferably, the ophthalmic angiogenesis-related disease is age-related macular degeneration.

Also disclosed is use of the FGFR-Fc fusion protein, the nucleic acid molecule encoding the protein, the vector comprising the nucleic acid molecule and/or a pharmaceutical composition comprising any one mentioned above in manufacture of a medicament for inhibiting angiogenesis. Also disclosed is use of the FGFR-Fc fusion protein, the nucleic acid molecule encoding the protein, the vector comprising the nucleic acid molecule and/or a pharmaceutical composition comprising any one mentioned above in manufacture of a medicament for the treatment or prevention of angiogenesis-related diseases, and preferably, the angiogenesis-related disease is a tumor or ophthalmic angiogenesis-related disease.

In view of different provisions for the subject protected in the patent systems of different countries, the disclosure has further provided the pharmaceutical uses corresponding to the methods mentioned above and the medicines for the intended uses. These various pharmaceutical uses and medicines are also covered in the protection scope of the present invention, as if they were already specifically described in the present disclosure.

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In the disclosure, only some specific embodiments claimed for protection are illustrated by way of example, in which the technical features described in one or more technical proposals can be combined with any one or more technical proposals, and these technical proposals obtained by combination are also covered in the protection scope of the application, as if these technical proposals obtained by combination were already specifically described in the disclosure.

With reference to the accompanying figures and the description in more detail below, the present invention will be illustrated by way of example only. It should be understood that the description below is only illustrated by way of example for the technical solutions claimed for protection by the present invention, and not regarded

as any limitation on these technical solutions. The protection scope of the present invention shall be defined by the claims as appended.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a structural representation of FGFR1-Fc fusion protein. FGFR1-Fc fusion protein is represented by a solid line, and the deleted amino acid is represented by a dash line; the antibody-like domain is represented by a circle; different antibody-like domains are represented by number 1-3; the disulfide bond is represented by s s; human IgG1 Fc is represented by a grey box; VEGFR1 signal peptide is represented by SP; the acidic box sequence is represented by a box with letter AB.

Fig. 2 shows the comparison of FGF-2 binding among various FGFR1-Fc fusion proteins. Binding of heparin (100 ng/mL) containing FGF-2 (50 ng/mL) or FGF-2 (50 ng/mL) alone to each FGFR1-Fc fusion protein (20 ng/mL) is detected by ELISA.

Fig. 3 shows SDS-PAGE of 26# FGFR1-Fc fusion protein.

Fig. 4 shows the binding of FGF-2 to a gradient concentration of 26# FGFR1-Fc fusion protein.

Fig. 5 shows the affinity between 26# FGFR1-Fc fusion protein and FGF-2.

Fig. 6 shows the effect of 26# FGFR1-Fc fusion protein on the HUVEC cell division induced by FGF-2.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions

Unless otherwise defined, all scientific terms used herein have the same meaning as commonly understood by those skilled in the art. With regard to the definitions and terms in the art, reference may be made to Current Protocols in Molecular Biology

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(Ausubel) by the skilled one. Standard three- and/or one-letter code used for expressing one of 20 common L-amino acids in the art are adopted as the abbreviation of amino acid residues.

Although the number ranges and approximate parameter values are given in a broad range in the present invention, all numbers in the specific examples are described as precise as possible. However, certain errors exist in any numerical values essentially, which may be resulted from the standard deviation during the measurement for each of them. Additionally, it should be understood that all ranges disclosed herein encompass any and all possible subranges contained therein. For example, it should be understood that the range "from 1 to 10" as described herein encompasses any and all possible subranges between the minimum 1 and the maximum 10 (including the endpoints); i.e., all subranges started from the minimum 1 or more, for example 1 to 6.1, and all subranges ended at the maximum 10 or less, for example 5.5 to 10. Additionally, it should be understood that any reference referred as "incorporated herein" is incorporated in its entirety.

Additionally, it should be noted that unless otherwise clearly and explicitly stated, the singular form includes the plural referent, as used in the present invention. The term "or" and the term "and/or" are used interchangeably, unless otherwise clearly indicated in the context.

In the claims which follow and in the description of the invention, except where the context requires otherwise due to express language or necessary implication, the word “comprise” or variations such as “comprises” or “comprising” is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

As used herein, the term "Fc", "Fc region", "Fc fragment" or "immunoglobulin Fc region" refers to the crystallizable fragment of immunoglobulin, and in the present invention, said Fc region is preferably the human IgG1 Fc region.

The term "Fc fusion protein" refers to the antibody-like molecule which incorporates the binding specificity of a heterologous protein and the effector function of a constant region of an immunoglobulin. In the term of molecular structure, a Fc fusion protein comprises the amino acid sequence having the required binding specificity and the sequence of a constant region of an immunoglobulin. A Fc fusion protein molecule generally comprises a binding site of a receptor or a ligand. The sequence of immunoglobulin constant region may be derived from any

immunoglobulin, for example, IgG-1, IgG-2, IgG-3 or IgG-4 subtype, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

The term "soluble" protein as used herein refers to the protein which may be dissolved in an aqueous solution at a biologically relevant temperature, pH level and osmotic pressure. The "soluble fusion protein" as used herein is intended to mean that the fusion protein does not contain a transmembrane region or an intracellular region.

As used herein, the term "isolated" refers to the following substance and/or entity: (1) which is isolated from at least some components which is present when initially produced (in natural environment and/or in a experiment device) and related thereto and/or (2) which is produced, prepared and/or manufactured artificially. The isolated substance and/or entity may be isolated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98%, about 99%, substantially 100% or 100% other components related to it initially.

The term "part" and "fragment" interchangeably refer to a part of polypeptide, nucleic acid or other molecular constructs.

The term "Ig-like domain" as used herein refers to immunoglobulin-like domain, which may be found in a plurality of protein families and involved in many biological functions, including cell-cell recognition, cell surface receptor, immune function and the like.

Fibroblast growth factor (FGF) is a heparin-binding growth factor family, which has 22 family members in the mammals (FGF 1-14, 16-23). FGF has many important biological functions, such as cell multiplication, differentiation, migration, angiogenesis and tumorigenesis. FGF exerts many biological functions by binding and activating the cell surface FGF receptor (FGFR). (See, for example, Eswarakumar *et al. Cytokine Growth Factor Rev.* 16: 139-149, 2005). Fibroblast growth factor receptor (FGFR) is the receptor that binds the family members of fibroblast growth factor. A part of fibroblast growth factor receptor is involved in the disease process. In the mammals, there are 4 FGFR genes: fgfR1-fgfR4. The fibroblast growth factor

receptor is composed of extracellular domain, transmembrane domain and intracellular domain. There are many members in FGFR family, which are different from each other in the term of ligand binding properties and kinase domains. However, the extracellular domains thereof are similar. There are three immunoglobulin-like (Ig-like) domains contained in their extracellular domains: the first Ig-like domain, the second Ig-like domain and the third Ig-like domain, and there is also a sequence contained between the first and the second Ig-like domain. Said sequence contained between the first and the second Ig-like domain is referred herein as the intermediate functional sequence region of the Ig-like domain of FGFR. Said intermediate regulation sequence comprises a region of acidic amino acids, referred as acidic box (AB).

As used herein, the term "the first Ig-like domain of FGFR" or "the first Ig-like domain" refers to the first Ig-like domain in the protein FGFR from the N-terminus, which has for example the amino acid sequence corresponding to position 40 to position 118 of SEQ ID NO: 1. Similarly, the term "the second Ig-like domain of FGFR" or "the second Ig-like domain" refers to the second Ig-like domain in the protein FGFR from the N-terminus, which has for example the amino acid sequence corresponding to position 163 to position 247 of SEQ ID NO: 1; the term "the third Ig-like domain of FGFR" or "the third Ig-like domain" refers to the first Ig-like domain in the protein FGFR from the N-terminus, which has for example the amino acid sequence corresponding to position 270 to position 359 of SEQ ID NO: 1. Preferably, the FGFR is FGFR1, and the first Ig-like domain of FGFR is the first Ig-like domain of FGFR1, and the second Ig-like domain of FGFR is the second Ig-like domain of FGFR1, and the third Ig-like domain of FGFR is the third Ig-like domain of FGFR1.

A part of sequence of hFGFR1 is given as follows, in which each Ig-like domain is shown in shaded area sequentially, see http://www.ncbi.nlm.nih.gov/protein/AAH15035.1

MWSWKCLLFWAVLVTATLCTARPSPTLPEQAQPWGAPVEVESFLVHPGDLL
QLRCRLRDDVQSINWLRDGVQLAESNRTRITGEEVEVQDSVPADSGLYACVT
SSPSGSDTTYFSVNVSDALPSSEDDDDDDDSSSEEKETDNTKPNPVAPYWTSP
EKMEKKLHAVPAAKTVKFKCPSSG180TPNPTLRWLKNGKEFKPDHRIGGYK
VRYATWSIIMDSVVPSDKGNYTCIVENEYGSINHTYQLDVVERSPHRPILQAG
LPANKTVALGSNVEFMCKVYSDPQPHIQWLKHIEVNGSKIGP300DNLPYVQI
LKTAGVNTTDKEMEVLHLRNVSFEDAGEYTCLAGNSIGLSHHSAWLTVLEAL
EER

The amino acid sequence of FGFR1 may be found in SEQ ID NO: 1, and its encoding nucleotide sequence may be found in SEQ ID NO: 4.

As used herein, the term "the intermediate functional sequence region of the Ig-like domain of FGFR" or "the intermediate functional sequence of the Ig-like domain of FGFR" or “IFS” refers to the sequence between the first Ig-like domain and the second Ig-like domain in the protein FGFR, and preferably, IFS sequence has the amino acid sequence corresponding to position 118 to position 162 of SEQ ID NO: 1. Unexpectedly, it has been found by the present inventor that there is a significant effect of the intermediate functional sequence region on the function of the Ig-like domain. In some embodiments of the present invention a FGFR fusion protein, which comprises a plurality of parts of various lengths derived from the intermediate functional sequence region, and particularly preferably, the part derived from the intermediate functional sequence region contains no acidic box. More preferably, the part derived from IFS has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162 or position 151 to position 162 of SEQ ID NO: 1. The protein FGFR is preferably FGFR1 (SEQ ID NO: 1), especially the protein human FGFR1. The amino acid sequence of the protein human FGFR1 may be found in SEQ ID NO: 1, and its cDNA sequence may be found in SEQ ID NO: 4.

The term "FGFR" as used herein refers to fibroblast growth factor receptor, which may be FGFR1, FGFR2, FGFR3 and/or FGFR4. Preferably, the FGFR in the present invention is FGFR1, more preferably, human FGFR1.

As used herein, the term "degenerate variant" is intended to mean that the degenerate variant comprises a degenerate change at the third position of the amino acid codon so that the degenerate variants encode the same amino acid, for example the wobble position of a triplet code comprising one or more changed variants (also referred as synonymous variant).

As used herein, the term "subject" refers to mammals, such as human. However, it may also be other animals, such as domesticated animals (such as dog and cat etc.), livestocks (such as cattle, sheep, pig and horse etc.) or experimental animals (such as monkey, rat, mouse, rabbit and guinea pig etc.).

As used herein, the term "percentage identity", "homology" or "identity" refers to the sequence identity between two amino acid sequences or nucleic acid sequences. The percentage identity may be determined by alignment between two sequences, and the percentage identity refers to the amount of the same residue (i.e., amino acid or nucleotide) at the same position in the sequence aligned. Sequence alignment and comparison may be performed using standard algorithms in the art (for example Smith and Waterman, 1981, *Adv. Appl . Math.* 2: 482; Needleman and Wunsch, 1970, *J. MoI. Biol.* 48: 443; Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci.*, USA, 85: 2444) or by the computerized versions of these algorithms (Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive, Madison, WI). Said computerized versions publicly available are BLAST and FASTA. Additionally, ENTREZ available through National Institutes of Health (Bethesda MD) may be used for sequence alignment. When BLAST and GAP-BLAST are used, default parameters for each program (for example, BLASTN, available on the website of National Center for Biotechnology Information) may be used. In one embodiment, the percentage identity between two sequences may be determined using GCG with a gap-weight of 1 so that the giving weight of each amino acid gap seems as if it is a single amino acid mismatch between two sequences. Alternatively, ALIGN (version

2.0), which is a part of GCG (Accelrys, San Diego, CA) Sequence Alignment Software Package, may be used.

As used herein, the term "hybridization" refers to the process by which a stable double-stranded polynucleotide is formed by non-covalent bonding between two single stranded polynucleotides. The term "hybridization" also may refer to triple-stranded hybridization. The double stranded polynucleotide (generally) produced is the "hybrid" or "duplex". "The condition for hybridization" generally includes a salt concentration lower than about 1 M, and more generally, lower than about 500 mM, and lower than about 200 mM. The hybridization temperature may be as low as 5°C, but it usually higher than about 22°C, and more usually higher than about 30°C, and preferably higher than about 37°C. Hybridization is usually carried out under strict conditions (i.e., the conditions under which the probe will hybridize to its target sequence). Strict hybridization conditions are dependent on the sequence and will be varied under different conditions. Higher hybridization temperature will be probably required by longer segments for specific hybridization. Since the hybridization stringency may be influenced by other factors (including base composition and length of the complementary strand, the presence of organic solvent and the degree of base mismatch), the combination of parameters is more important than the absolute value of any single parameter. Generally, the strict condition is selected as 5°C lower than the Tm of the sequence under certain ionic strength and pH. Exemplary strict conditions include pH 7.0 to 8.3, sodium ion (or other salts) concentration of at least 0.01 M to no more than 1 M and temperature of at least 25°C. For strict conditions, see, for example Sambrook, Fritsche and Maniatis. “Molecular Cloning A laboratory Manual”, 2nd edition, Cold Spring Harbor Press (1989) and Anderson “Nucleic Acid Hybridization”, 1st edition, BIOS Scientific Publishers Limited (1999), which are incorporated herein by reference for all purposes mentioned above.

As used herein, the term "linker", "peptide linker", "linking sequence" or "linker sequence" refers to a short amino acid sequence by which individual domain and/or region involved in the present fusion protein are linked together, and the length of the short amino acid sequence is generally 0-20 amino acids, and preferably, 2-10 amino acids.

As used herein, the term of "the amino acid sequence corresponding to SEQ ID NO: N" in a fusion protein or part or domain is intended to mean said fusion protein or part or domain has the amino acid sequence substantially as indicated by SEQ ID NO: N, and preferably, containing no more than 1, 2, 3, 4, 5, 10 or 20 substitutions, additions and deletions of amino acids, and yet preferably, said fusion protein or part or domain shares at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with the amino acid sequence of SEQ ID NO: N, and more preferably, said fusion protein or part or domain has the amino acid sequence as indicated by SEQ ID NO: N.

As used herein, the term "FGFR-Fc fusion protein" refers to a fusion protein which comprises the part derived from the extracellular domain of FGFR and the part derived from the immunoglobulin Fc region, wherein the part derived from the extracellular domain of FGFR may: (1) comprise the amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity, with the amino acid sequence indicated by any one of SEQ ID NOs: 9-15 or composed thereof; (2) comprise the amino acid sequence encoded by the nucleotide sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity, with the nucleotide sequence indicated by any one of SEQ ID NOs: 16-22 or composed thereof; or (3) possess the amino acid sequence indicated by any one of SEQ ID NOs: 9-15, or the amino acid sequence encoded by the nucleotide sequence indicated by any one of SEQ ID NOs: 16-22.

In some preferable embodiments, the FGFR-Fc fusion protein may be encoded by the nucleic acid, in which the nucleotide sequence encoding the part derived from the extracellular domain of FGFR comprises the sequence of which the complementary

sequence is hybridized with the nucleotide sequence as indicated by any one of SEQ ID NOs: 16-22 under stringent conditions, or comprises the degenerative variant of the nucleotide sequence as indicated by any one of SEQ ID NOs: 16-22. In some preferable embodiments, the nucleotide sequence encoding the immunoglobulin Fc region comprises the sequence of which the complementary sequence is hybridized with the nucleotide sequence indicated by SEQ ID NO: 8 under stringent conditions, or comprises the degenerative variant of the nucleotide sequence indicated by SEQ ID NO: 8.

In other preferable embodiments, the FGFR-Fc fusion protein includes the FGFR-Fc fusion protein variant. In one embodiment, the variant includes the variant which contains no more than 2, 3, 4, 5 or 10 substitutions, additions or deletions of amino acid in the part derived from IFS corresponding to the amino acid sequence indicated by position 134 to position 162, position 145 to position 162 or position 151 to position 162 of SEQ ID NO: 1, and preferably, the variant retains the angiogenesis-inhibitory capacity. In another embodiment, the variant includes the variant which contains no more than 2, 3, 4, 5, 10 or 20 substitutions, additions or deletions of amino acid in D2 domain corresponding to the amino acid sequence indicated by position 163 to position 247 of SEQ ID NO: 1, and preferably, the variant retains the angiogenesis-inhibitory capacity. In another embodiment, the variant includes the variant which contains no more than 2, 3, 4, 5, 10 or 20 substitutions, additions or deletions of amino acid in D3 domain corresponding to the amino acid sequence indicated by position 270 to position 359 of SEQ ID NO: 1, and preferably, the variant retains the angiogenesis-inhibitory capacity. In another embodiment, the substitution, addition or deletion is located at the linker or the linking part.

In addition to the naturally occurring modifications in the part derived from the extracellular domain of FGFR and the part derived from immunoglobulin Fc region, other post-translational modifications may also be comprised in the FGFR-Fc fusion

protein. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, esterification and acylation. As a result, non-amino acid component may be comprised in the modified FGFR-Fc fusion protein, for example polyethylene glycol, lipid, polysaccharide or monosaccharide, and phosphoric acid. Effect of such non-amino acid components on the function of the FGFR-Fc fusion protein may be tested as described for other FGFR-Fc fusion protein variants herein. When FGFR-Fc fusion protein is produced in a cell, post-translational processing is also possibly important for correct folding and/or protein function. Special cell machines and unique mechanisms exist in different cells (for example CHO, HeLa, MDCK, 293, WI38, NIH-3T3 or HEK293) for these post-translational activities, and different cells may be selected to make sure correct modification and processing of FGFR-Fc fusion protein.

The fusion protein as described herein may be produced by any method known in the art. For example, it may be produced by chemical synthesis or from nucleic acid expression. The peptides used in the present invention may be easily prepared according to the established standard liquid, or preferably, solid phase peptide synthesis method known in the art (see, for example J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford, Illinois (1984), in M. Bodanzsky, and A. Bodanzsky, The Practice of Peptide Synthesis, Springer Verlag, New York (1984)). The fusion protein may be produced by the techniques known in the art so that one or more intramolecular crosslinkings may be formed between the cysteine residues located in the polypeptide sequence expected to be comprised in the protein (see, for example US patent No. 5478925). In addition, general modifications may be performed to the protein described herein by adding cysteine or biotin to the C-terminus or N-terminus of the protein.

As used herein, "therapeutically effective amount" or "effective amount" refers to the dosage which is sufficient to show the benefit to the subject administrated. The actually administrated dosage, the rate and the time course of administration are

dependent on the condition of the patient and the severity of the disease. Finally, the physician is responsible for the prescription (for example decision on the dosage etc.) and will make a decision for the treatment, usually by considering the disease treated, individual condition of the patient, position of delivery, the method for administration and other factors known to the physician.

A series of isolated soluble FGFR-Fc fusion proteins are constructed by the present inventor, which may bind FGF and effectively inhibit the cell division induced by FGF. The fusion protein preferably comprises: the part derived from IFS, D2, D3 and immunoglobulin Fc region.

Unexpectedly, it has also been found by the present inventor that the binding of FGF by the fusion protein is significantly influenced by the length of the part derived from IFS. Therefore, in some embodiments of the present invention fusion proteins comprising the parts derived from IFS with various lengths. Preferably, the part derived from IFS comprises no acidic box, and more preferably, it has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162 or position 151 to position 162 of SEQ ID NO: 1. In some preferable embodiments, the part derived from IFS comprises the fusion protein corresponding to the amino acid sequence indicated by position 145 to position 162 of SEQ ID NO: 1, which has extremely high FGF affinity and may particularly effectively inhibit the cell division induced by FGF.

In some embodiments of the present invention, a soluble FGFR-Fc fusion protein is provided, which comprises: D1, a part derived from IFS, D2, D3 and immunoglobulin Fc region. Preferably, the part derived from IFS comprises no acidic box, and more preferably, it has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162 or position 151 to position 162 of SEQ ID NO: 1.

In some other embodiments of the present invention, a soluble FGFR-Fc fusion protein is provided, which comprises: a part of D1, a part derived from IFS, D2, D3

and immunoglobulin Fc region. Preferably, the part derived from IFS comprises no acidic box, and more preferably, it has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162 or position 151 to position 162 of SEQ ID NO: 1.

In some other embodiments of the present invention, a soluble FGFR-Fc fusion protein is provided, which is composed of: a part derived from IFS, D2, D3 and immunoglobulin Fc region. Preferably, the part derived from IFS comprises no acidic box, and more preferably, it has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162 or position 151 to position 162 of SEQ ID NO: 1.

In some other embodiments of the present invention, a soluble FGFR-Fc fusion protein is provided, which is sequentially composed of, from the N-terminus to the C-terminus, a part derived from IFS, D2, D3 and immunoglobulin Fc region. Preferably, the part derived from IFS comprises no acidic box, and more preferably, it has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162 or position 151 to position 162 of SEQ ID NO: 1.

In some other embodiments of the present invention, an FGFR-Fc fusion protein is provided, which may inhibit tumor cells directly or indirectly. Preferably, the FGFR-Fc fusion protein of the present invention inhibits tumor cells directly. More preferably, the growth of tumor cells is inhibited by the FGFR-Fc fusion protein of the present invention by at least 10%, 20%, 30%, 40%, 50%, 80%, 90% and 95% etc. The tumor cells may be any tumor cells, for example, leukaemia, lung cancer, liver cancer, head and neck cancer, stomach cancer, bladder cancer, carcinoma of uterine cervix etc. Particularly, the inhibition is achieved by direct binding to tumor cells.

In some embodiments, the present invention includes use of (i) FGFR-Fc fusion protein, or (ii) the polynucleotide encoding such fusion protein, in the preparation of the compositions or medicaments for the treatment of diseases mediated by or related to angiogenesis. For example, in one embodiment, the present invention includes use

of (i) FGFR-Fc fusion protein, or (ii) the polynucleotide encoding such fusion protein in the preparation of the medicaments as an angiogenesis inhibitor.

In some embodiments, the FGFR-Fc fusion protein according to the present invention may be produced by the expression of the nucleotide sequence as indicated by any one of SEQ ID NOs: 16-22 in a mammalian cell line. In particular, the mammalian cell line is CHO cell line.

Additionally, in the present invention, the FGFR-Fc fusion protein as described below is provided, in which a part derived from the extracellular domain of FGFR may be fused with the immunoglobulin Fc region with or without a linker.

In some other embodiments, the present invention includes the isolated nucleic acid molecules encoding the FGFR-Fc fusion protein, and the present invention also includes use of these molecules in manufacture of a medicament. The nucleic acid may be recombinant, synthetic or produced by any available methods in the art, and the method includes cloning by means of using a standard technique.

In some other embodiments, the present invention includes a vector comprising the isolated nucleic acid molecule of the present invention. The vector may be an expression vector, in which the nucleic acid is operatively linked to a control sequence which is able to provide the expression of the nucleic acid in a host cell. A plurality of vectors may be used. For example, suitable vectors may include virus (for example poxvirus, adenovirus, baculovirus etc.); yeast vector, bacteriophage, chromosome, artificial chromosome, plasmid, cosmid.

In some embodiments, the present invention further includes the cells transfected by these vectors so that the FGFR-Fc fusion protein is expressed. The host cell suitable for the present invention may be prokaryotic cell or eukaryotic cell. They include bacteria, for example *E. coli*, yeast, insect cell and mammalian cell. The mammalian cell lines that may be used include, but are not limited to, Chinese Hamster Ovary (CHO) cell, baby hamster kidney cell, NS0 mouse myeloma cell, monkey and human cell lines, and derivate cell lines thereof, etc.

In another aspect of the present invention, a method for angiogenesis inhibition is provided, comprising administrating the FGFR-Fc fusion protein of the present invention to the subject in need thereof. Preferably, the method is carried out in the mammals.

In another aspect of the present invention, a method for binding FGF *in vitro* or *in vivo* is provided, which comprises contacting FGF to the fusion protein according to the present invention.

In another aspect of the present invention, a method for the treatment or prevention of tumors in the mammals is provided, which comprises administrating the FGFR-Fc fusion protein of the present invention to the subject in need thereof, and preferably, the tumor is a solid tumor.

In another aspect of the present invention, a method for the treatment or prevention of ophthalmic angiogenesis-related diseases in the mammals is provided, which comprises administrating the FGFR-Fc fusion protein of the present invention to the subject in need thereof, and preferably, the ophthalmic angiogenesis-related disease is age-related macular degeneration.

The present invention also relates to use of the FGFR-Fc fusion protein in the preparation of medicaments for angiogenesis inhibition. Additionally, the present invention also relates to use of the FGFR-Fc fusion protein in the preparation of medicaments for the treatment or prevention of angiogenesis-related diseases, and preferably, angiogenesis-related diseases are tumors or ophthalmic angiogenesis-related disease.

The angiogenesis-related diseases as described in the present invention include, but are not limited to, angiogenesis-dependent cancers, comprising, for example, solid tumor, hematogenic tumor (for example leukaemia) and tumor metastasis; benign tumor, for example, angioma, acoustic neuroma, neurofibroma, trachoma and pyogenic granuloma; rheumatoid arthritis; psoriasis; rubeosis; Osler-Webber

Syndrome; myocardial angiogenesis; plaque neovascularization; telangiectasia; hemophiliac joint and angiofibroma.

In some embodiments of the methods described, one or more FGFR-Fc fusion proteins may be administrated together (simultaneously) or at a different time (sequentially). Additionally, the fusion protein may be administrated together with additional medicament used for cancer treatment or angiogenesis inhibition.

In some embodiments, the method disclosed in the present invention may be used alone. Alternatively, the subject method may be combined with other conventional anticancer therapies for the treatment or prevention of proliferative diseases (for example tumor). For example, these methods may be used for the prevention of cancers, the prevention of cancer relapse and postoperative metastasis, and may be used as a supplement for other cancer therapies. As disclosed in the present invention, the effectiveness of conventional cancer therapies (for example, chemotherapy, radiotherapy, phototherapy, immunotherapy and operation) may be enhanced by using target polypeptide therapeutic agents.

In ophthalmology, angiogenesis is related to, for example, diabetic retinopathy, retinopathy of prematurity, age-related macular degeneration, corneal transplantation rejection, neovascular glaucoma and RLF (retrolental fibroplasia). The FGFR-Fc fusion protein disclosed herein may be administrated inside the eye or by other routes. Other diseases related to angiogenesis in ophthalmology include, but not limited to, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogren, acne rosacea, phlyctenosis, syphilis, Mycobacteria infection, lipid degeneration, chemical burn, bacterial ulcer, fungal ulcer, Herpes simplex infection, Herpes zoster infection, protozoan infection, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, mariginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, periphigoid radial keratotomy and comeal graph rejection, sickle cell anemia, sarcoid, pseudoxanthoma

elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosis, retinopathy of prematurity, Eales disease, Bechets disease, infection resulting in retinitis or choroiditis, presumed ocular histoplasmosis, Bests disease, myopia, optic pit, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complication.Other diseases include, but not limited to, rubeosis (neovascularization of the angle) related diseases and diseases induced by abnormal hyperplasia of the fibrous blood vessel or fibrous tissue, including all kinds of proliferative vitreoretinopathy.

Administration

The fusion protein of the present invention may be administrated alone, but preferably, as a pharmaceutical composition which usually comprises a suitable pharmaceutical excipient, diluent or carrier selected according to the intended administration route. The fusion protein may be administrated to the patient in need thereof by any suitable route. A precise dosage will be dependent on many factors, including exact properties of the fusion protein.

Some suitable administration routes include (but are not limited to) oral, rectal, nasal, topical (including buccal and sublingual), subcutaneous, vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intracutaneous, intrathecal and extradural) administration.

For intravenous injection and injection at the focal site, active ingredients are present in the form of a parenterally-acceptable aqueous solution, which is free of pyrogen and has appropriate pH value, isotonicity and stability.

A suitable solution may be well formulated by the skilled one in the art using, for example, isotonic excipients such as sodium chloride injection, Ringer's injection, Ringer's lactate injection. As required, preservative, stabilizer, buffering agent,

antioxidant and/or some other additives may be added. The pharmaceutical composition orally administrated may be in a form of tablet, capsule, powder or oral liquid etc. Solid carrier, such as gelatin or adjuvant, may be comprised in a tablet. Liquid pharmaceutical composition usually comprises liquid carrier, such as water, petroleum, animal or vegetable oil, mineral oil or synthetic oil. Also included may be normal saline solution, glucose or other sugar solutions or glycols such as ethylene glycol, propylene glycol or polyethylene glycol.

Examples of the techniques and schemes as mentioned above and other techniques and schemes as used according to the present invention may be found in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A. (ed), 1980.

Cloning of the fusion protein and construction of the expression plasmid

The FGF receptor fragment are obtained from the amplification of the cDNA template of corresponding receptor through PCR, and IgG1 Fc fragment is obtained from the cDNA amplification of the human-derived IgG1 through PCR. When PCR primers are designed, linking sequences are introduced between different fragments so that these different fragments may be finally linked by overlap PCR to form reading frames for different fusion proteins, and endonuclease BspE I and Pst I site are added to both ends of the cDNA. The cDNAs for different fusion proteins may be cloned to the expression plasmid after digestion by BspE I and Pst I. The plasmid after cloning may be determined by endonuclease digestion, electrophoresis and finally DNA sequencing.

Expression and purification of the fusion protein

The present fusion protein may be expressed and purified by techniques commonly used in the art. DNA from corresponding fusion protein plasmid was purified using plasmid purification kit (MAX) available from Qiagen, and the

concentration of plasmid DNA was determined using UV spectrophotometry, and the plasmid was transfected to CHO cell using FUGENE 6 liposome (Roche). Specific method for transfection was performed according to the specification of the product. Based on the expression amount required for the proteins, two methods were employed in the present invention for protein expression: (1) transient expression, in which the fusion protein contained culture supernatant was usually harvested 48-72 h after transfection, and the relative content of the fusion protein was then determined using human IgG ELISA so that the fusion protein may be rapidly and efficiently obtained; (2) establishing a stable cell line and producing the common DHFR-defective CHO cell expression system using the recombinant protein medicament expression, the basic process of which includes cell transfection, selection of stably transfected cell, clone screening, stress amplification, culture medium and process optimization etc., and finally realizing a large-scale suspension culture of CHO engineering cell strain in a serum free culture medium. The culture product was collected and the fusion protein was purified using Protein A affinity column. The purified protein was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and subsequently all eluates in which the required expression product was contained were combined and filtered using a 0.22 μm filter, and then protein quantification was carried out according to a plurality of methods such as Lowry protein assay. The volume of CHO cell culture in the present invention was at a level of 10 L bioreactor, through which the fusion protein obtained after purification could satisfy the protein amount required in the animal experiments, and also a basis was established for future scaling-up.

Neutralization of FGF by the fusion protein was validated at a level of protein

After the fusion protein expressed by CHO was obtained, the binding capacity of the fusion protein to FGF is evaluated in the present invention at a level of protein. Binding experiment and affinity experiment were performed for validation in the

present invention, in which steps of the binding experiment included: after initially coated by FGF-2 on a 96-well ELISA plate, the coated well was blocked by BSA followed by adding each fusion protein at the same concentration, and then a secondary antibody to human IgG Fc-HRP was added after washing, and the samples were developed, stopped and read at 450nm on a ELISA plate, and finally the fusion protein which had binding capacity to FGF-2 was screened based on the signal strength. The affinity experiment was performed in order to determine the affinity of the fusion protein to FGF-2 in the solution system, which comprised the following steps: FGF-2 was initially coated on a 96-well ELISA plate to capture the antibody, and then the coated well was blocked by BSA, and subsequently a mxiture of the fusion protein and FGF-2 which was previously prepared and incubated were added with a gradient of diluted standards, and after incubation, an HRP-labeled detection antibody was added (using antibody 2 which specifically detected free VEGF or FGF-2), and subsequently the samples were developed, stopped and read at 450nm on a ELISA plate, and finally the relative concentration of free FGF-2 was detected in the mixture of the fusion protein and FGF-2. Through the experiments above, the fusion protein having a blocking effect on FGF-2 was screened.

Neutralization of FGF by the fusion protein was validated at a cellular level

After the binding capacity of the fusion protein to FGF-2 was determined at a level of protein, its angiogenesis-inhibiting effect will be further validated at a cellular level in the present invention. The inhibition capacity of the fusion protein on the division and migration of the vascular endotheliocyte is examined by the division test using human umbilical vein endothelial cell (HUVEC) and the HUEVC cell migration test. The inhibition capacity of the fusion protein on the division of HUVEC cell can be examined by the HUVEC cell division test, which comprises the following steps during the experiment: 3000 HUVEC cells/well were inoculated to a 96-well plate and cultured at 37°C in an incubator supplemented with 5% CO_2, and then FGF-2 as

well as a mixture of the fusion protein at different concentrations with FGF-2 are added respectively, and after culturing for another 3-4 days, 10% CCK-8 is added and cultured for 2 h before the sample is read at 450 nm on a ELISA plate. The inhibition capacity of the fusion protein on the division of vascular endotheliocyte induced by FGF-2 was evaluated based on the difference of absorbance, and the median effective concentration of the fusion protein was obtained for FGF-2 inhibition. The inhibition capacity of the fusion protein on HUVEC cell migration was examined by the HUVEC cell migration test, which comprises the following steps during the experiment: 50000 HUVEC cells as well as the fusion protein at various concentrations were initially inoculated in the upper chamber, while 600 μL FGF-2 containing culture liquid was added into the lower chamber, and subsequently, the sample was cultured at 37°C in an incubator supplemented with 5% CO_2 for 20-24 h before cells on the face side of the membrane of the upper chamber were removed, and then cells on the back side of the membrane were fixed, stained and washed with PBS before observed and counted under an inverted microscope. The migration of HUVEC cells induced by the stimulation of FGF-2 was demonstrated by counting the HUVEC cells on the back side of the membrane, and the inhibition capacity of the fusion protein on the migration of the vascular endotheliocyte was tested by adding the fusion protein at various concentrations into the culture liquid. Through the experiments mentioned above, the inhibition capacity of the new fusion protein constructed in the present invention was validated on the division and migration of the vascular endotheliocyte induced by FGF-2, which also provided a basis for future animal experiments.

Tumor growth-inhibiting capacity of the fusion protein was validated by the tumor model

After the blocking effect of the new fusion protein in the present invention on FGF-2 signal was demonstrated by experiments at a protein level and a cellular level,

its anti-tumor capacity would be tested in animal tumor models in the present invention. In the present invention, the anti-angiogenesis and anti-tumor effect of the fusion protein would be validated by models commonly used in searching medicaments for angiogenesis and tumor, for example, LLC mouse lung cancer, U87 gliocytoma, B16 melanoma and so on. In animal experiments, in addition to conventional control groups, control medicaments, such as VEGF-Trap, FP-1039, would also be included so as to obtain comparative data for anti-tumor capacity. During experiments, 100 μL tumor cell liquid with appropriate amount was subcutaneously injected into C57 mouse on one side of the back, and the tumor volume was measured with a vernier caliper twice a week. Upon the tumor grew to about 200 mm^3, the fusion protein at various concentrations was subcutaneously injected and the mice were sacrificed after 2-3 weeks. Subsequently, the tumor volume was measured with a vernier caliper, and the anti-tumor effect of the fusion protein was validated by the size of the tumor. Furthermore, individual tumor tissue was analyzed using methods such as immunohistochemistry to investigate the regulation mechanism of angiogenesis.

Examples

Example 1: Construction of recombinant expression plasmid for FGFR1-Fc fusion protein

The FGF receptor fragment is obtained from the amplification of the cDNA templet of FGF receptor through PCR, and IgG1 Fc fragment is obtained from the cDNA amplification of the human-derived IgG1 through PCR. A commercially available cDNA (PCR Ready First Strand cDNA, derived from human adult colon cancer tissue, BioChain) was used as the template for FGFR1 fragment. Total RNA was extracted from the blood of healthy human subjects using human blood RNA extraction kit (QIAGEN). According to the manufacturer's instruction of reverse transcription kit (Promega), RT-PCR was performed using M-MLV reverse

transcriptase (Promega) so that RNA was reversely transcripted to cDNA which was used as the template for IgG1 Fc fragment. RT-PCR was performed according to the manufacturer's instruction of reverse transcription kit, which has the following steps: Oligo dT, dNTP, total RNA and DEPC H2O were mixed homogeneously and reacted at 70°C for 10 min before placed on ice for 5 min, and subsequently RNase inhibitor, M-MLV reverse transcriptase and reaction buffer were added. The mixture was reacted at 42°C for 1 h and subsequently at 70°C for 15 min, and the cDNA obtained may be used as the template.

Various FGFR1 fragments were individually amplified by PCR using the cDNA from human adult colon cancer tissue as the template (the primers were listed in table 1), and IgG1 Fc fragment was amplified by PCR using human blood cDNA as the template (the primers were listed in table 1 and 2). The reaction conditions for the PCR were as follows: 5 min of pre-denaturalization at 98°C, total 30 cycles of 30 s of denaturalization at 98°C, 45 s of annealing at 56°C and 2 min of extension at 72°C, and finally another 10 min of extension. When PCR primers were designed, 20 or more complementary base sequences were introduced as the linking sequence between FGFR1 fragment and IgG1 Fc fragment so that the FGFR1 fragment and IgG1 Fc fragment may be subsequently linked by overlap PCR to form reading frames for different fusion proteins, and at the same time, restriction endonuclease BspE I and Pst I site were added at both ends of the PCR product.

Subsequently, overlap PCR was carried out to obtain each FGFR1-Fc fusion protein fragment by amplification. The process of the overlap PCR reaction may be divided into two rounds, in which the fragment required for linking and containing no primer was included in the first round with reaction conditions as follows: 5 min of pre-denaturalization at 98°C, 6 cycles of 30 s of denaturalization at 98°C, 45 s of annealing at 56°C and 5 min of extension at 72°C, and finally another 10 min of extension at 72°C; after the first round, the second round of PCR was carried out by adding the primers for both ends with reaction conditions as follows: 5 min of

pre-denaturalization at 98°C, 30 cycles of 30 s of denaturalization at 98°C, 45 s of annealing at 56°C and 2 min of extension at 72°C, and finally another 10 min of extension at 72°C; through the process above, reading frames for different fusion proteins were spliced, and at the same time, restriction endonuclease BspE I and Pst I site were added at both ends of the cDNA.

After amplification, the fragments amplified by PCR were purified using QIAquick PCR purification kit (QIAGEN). cDNAs of various fusion proteins and the eucaryotic expression plasmid pSV2-dhfr (ATCC) were digested by BspE I and Pst I, respectively. Subsequently, 1% agarose gel electrophoresis was performed on the digested samples under a voltage of 90 V. Target fragments were recovered using QIAquick gel extraction kit (QIAGEN) before ligating at 16°C for 1 h using a ligase (NEB). The mixture for ligation reaction was transformed to the competent Top10 *E. coli* under the conditions of 90 s of reaction at 42°C followed by 3 min of standing on ice. After the sterile LB culture broth (free of antibody) added, the mixture was shaken at 250 rpm in a shaker at 37°C for 1 h before coating on a LB plate supplemented with ampicillin. The plate was cultured overnight in a thermostated incubator at 37°C, and then single colonies were picked out and transferred to an ampicillin-containing LB culture broth. The inoculated culture broth was shaken at 250 rpm in a shaker at 37°C overnight before the plasmid was extracted using alkaline lysis. Subsequently, the sample was digested by restriction endonuclease before evaluated by 1% agarose gel electrophoresis under a voltage of 90 V. The recombinant plasmid with correct endonuclease digestion was confirmed by DNA sequencing. Based on the steps above, 19#, 13#, 22#, 23#, 26#, 29# and 8# expression plasmid for FGFR1-Fc fusion protein were constructed. The protein sequence of FGFR1-Fc in each fusion protein and its encoding nucleotide sequence were listed in Table 3. The schematic diagram of the fusion protein structure was shown in Fig. 1.

Table 1: Primers used for amplification of FGFR1 fragment

Fusion	Upstream primer	Downstream primer

protein		
19#	19#-FGFR1For（SEQ ID NO: 24） TAGTTCCGGAAGGCCGTCCCCGACCTTGCCTG	FGFR1Rev（SEQ ID NO: 31） GTTTTGTCCTCCAGGTAC AGGGGCGAGGTC
13#	13#-FGFR1For（SEQ ID NO: 25） TAGTTCCGGAAAAAATCGCACCCGCATCACAG	FGFR1Rev
22#	22#-FGFR1For（SEQ ID NO: 26） TAGTTCCGGAGTAACCAGCAGCCCCTCGGGC	FGFR1Rev
23#	23#-FGFR1For（SEQ ID NO: 27） TAGTTCCGGATCCTCTTCAGAGGAGAAAGAAAC	FGFR1Rev
26#	26#-FGFR1For（SEQ ID NO: 28） TAGTTCCGGAAAACCTAACCCCGTAGCTCCAT	FGFR1Rev
29#	29#-FGFR1For（SEQ ID NO: 29） TAGTTCCGGACCATATTGGACATCCCCAGAAAAG	FGFR1Rev
8#	8#-FGFR1For（SEQ ID NO: 30） CTAGCTCCGGACCAGAAAAGATGGAAAAGAAATTGC	FGFR1Rev

Table 2: Primers used for amplification of IgG1 Fc fragment

	Upstream primer	Downstream primer
IgG1 Fc fragment	FcFor（SEQ ID NO: 32） CTGTACCTGGAGGACAAAACTCACACATGC	FcRev（SEQ ID NO: 33） GATATCTGCAGTCATTT ACCCGGAGACAGG

Table 3: Protein sequences and nucleotide sequences for FGFR1-Fc fusion proteins

Fusion protein	Upstream primer	Downstream primer

19#	SEQ ID NO: 9	SEQ ID NO: 16
13#	SEQ ID NO: 10	SEQ ID NO: 17
22#	SEQ ID NO: 11	SEQ ID NO: 18
23#	SEQ ID NO: 12	SEQ ID NO: 19
26#	SEQ ID NO: 13	SEQ ID NO: 20
29#	SEQ ID NO: 14	SEQ ID NO: 21
8#	SEQ ID NO: 15	SEQ ID NO: 22

Example 2: Transient expression and quantification of the fusion proteins

The DNA of individual fusion protein plasmid was purified using MAX Plasmid Purification Kit (Qiagen). The concentration of the plasmid DNA was determined by UV spectrophotometry. 1 μg recombinant plasmid and 6 μL liposome (FuGENE 6 Transfection Reagent, Roche) were homogeneously mixed into 100 μL fresh IMDM culture broth (GIBCO); after standing for 15 min, the mixture was added to the CHO cells (ATCC) cultured overnight after inoculation at a cell density of 3×10^5/mL into a 6-well plate; the mixture was cultured at 37°C in an incubator supplemented with 5% CO_2 for 48 h with a cell complete culture broth (IMDM medium containing 10% FBS, 1% HT and 1% glutamine, all supplied by GIBCO); subsequently, the supernatant was collected and determined for the relative content of the fusion protein using human IgG ELISA kit for protein quantification (BETHYL). The relative content of the fusion protein expressed and secreted by CHO was determined with the following steps: 100 μL anti-human IgG-Fc protein (10 μg/mL) purified by affinity was coated to a 96-well ELISA plate (IMMULON) and subsequently washed for 5 times using 300 μL PBST washing solution; each coated well was blocked with 200 μL freshly prepared blocking working solution (blocking stock solution : PBS=1: 19) and incubated at 37°C for 1 h; after washed in 300 μL PBST washing solution for 5 times, 100 μL IgG solution diluted in a gradient (200 ng/mL original concentration and

diluted by PBS in the multiple proportion of 1: 2) as a standard and 100 μL culture supernatant of each fusion protein diluted in a gradient (starting with the concentration of each culture supernatant, and diluted by PBS in the multiple proportion of 1: 5) were added to each well and incubated at 37°C for 2h; after washed in 300 μL PBST washing solution for 5 times, 100 μL anti-human IgG Fc-HRP secondary antibodies diluted with PBS in a ratio of 1: 10000 was added and incubated at 37°C for 1h; after washed, the well was developed by adding 100 μL developing solution (KPL); finally, after the development was stopped by adding 100 μL stopping solution (KPL), the absorbance of the ELISA plate was read at a wavelength of 450 nm on a ELISA reader. The concentrations of various fusion proteins may thereby be determined according to the standard curve.

Example 3: Binding of the fusion proteins

The binding capacity of 19#, 13#, 22#, 23#, 26#, 29# and 8# fusion protein constructed above to FGF-2 was detected by ELISA. Initially, a 96-well ELISA plate (IMMULON Company) was coated by 100 μL solution containing 50 ng/mL FGF-2 (R&D Systems) as well as containing 100 ng/mL heparin (Sigma Company) and 50 ng/mL FGF-2. Subsequently, the plate was washed by 300 μL PBST washing solution for 5 times before each coated well was blocked by 200 μL freshly prepared blocking working solution (KPL Company) (blocking stock solution : PBS = 1:19) and incubated at 37°C for 1 h. After washed in 300 μL PBST washing solution for 5 times, 100 μL solutions of various fusion proteins (dissolve in PBS, pH=7.2, concentration of 20 ng/ml) were added and incubated at 37°C for 2 h. After washed in 300 μL PBST washing solution for 5 times, 100 μL secondary antibody to human IgG Fc-HRP (BETHYL Company) diluted with PBS in a ratio of 1:10000 was added and incubated at 37°C for 1h. After washed in 300 μL PBST washing solution for 5 times, the well was developed to the presence of color at room temperature in a dark place by adding 100 μL developing solution (KPL Company), and finally the development was

stopped by adding 100 μL stopping solution (KPL Company) before the absorbance of the ELISA plate was read at a wavelength of 450 nm on a ELISA reader. The higher the binding capacity of the fusion protein to FGF2 was, the larger the absorbance was and the stronger the signal was. Based on the strength of the signal, 26# fusion protein was determined to have the highest binding capacity to FGF-2. Comparison of FGF-2 binding among various fusion proteins was shown in Fig. 2. It can be seen from Fig. 2 that 19#, 13#, 22#, 23#, 26# and 29# fusion protein constructed in the present invention bound to FGF at different extents in the presence of heparin, and particularly, the binding extent of 23#, 26# and 29# was extremely higher than control, and higher than that of 19#, 13# and 22#, indicating that the fusion proteins containing no acidic box according to the present invention had excellent effect. Among others, especially high binding extent was demonstrated by 26#, which presented us a clue that the fusion protein of the present invention had significantly better binding effect when comprises a part of certain length derived from the intermediate functional sequence of the Ig-like domain of FGFR.

Example 4: Stable expression and purification of the fusion proteins

DHFR-defective CHO cells (ATCC) were transfected by the recombinant expression plasmid of 26# fusion protein (possessing a high FGF-2 binding capacity) through a liposome (Roche). Particularly, 5 μg recombinant plasmid and 30 μL liposome (FuGENE 6 Transfection Reagent, Roche) were homogeneously mixed into 100 μL fresh IMDM culture broth (GIBCO); after standing for 15 min, the mixture was added to the DHFR-defective CHO cells (ATCC) cultured overnight after inoculation at a cell density of 3×10^5/mL in a 10 cm culture dish (Corning); the mixture was cultured at 37°C in an incubator supplemented with 5% CO_2 for 2-3 days with a cell complete culture broth containing 10% FBS, 1% HT and 1% glutamine in a IMDM culture medium (all supplied by GIBCO); subsequently, the cells were digested by trypsin (GIBCO), inoculated at a cell density of 3×10^5/mL in 30 mL

serum-free 302 culture medium (SAFC) in a flask, and selectively cultured at 37°C in an incubator supplemented with 5% CO_2 at 100 rpm to a cell density of 10^6/mL. Subsequently, 3000 cells were inoculated into a 10 cm culture dish (Corning) (the culture broth containing 10% FBS and 1% glutamine in an IMDM culture medium) and cultured at 37°C in an incubator supplemented with 5% CO_2 to form single clones. These single clones were picked out and cultured in a 96-well plate (Corning). The relative content of the fusion protein expressed and secreted by each individual single clone was determined using a human IgG ELISA kit for protein quantification (BETHYL) under the same conditions and steps as described in Example 2 for the determination of the relative content of the fusion protein. The clone with the highest expression amount was screened out and transferred to a 6-well plate for culturing to a confluence rate of about 70%. The cells were digested by trypsin and transferred to a 10 cm culture dish. Subsequently, gradual stress amplification was carried out by adding methotrexate (MTX, Sigma) with various concentrations (10 nM, 20 nM, 50 nM, 100 nM, 200 nM and 500 nM). After stress amplification, the cells were digested by trypsin and inoculated at a cell density of 3×10^5/mL in a flask. The expression amount of a single cell was determined so that genetically engineered stains of CHO were obtained for expressing a particular fusion protein. Finally, large-scale suspension culture (volume of 10L) of the genetically engineered stain of CHO was carried out at 37°C, 5% CO_2, 40% dissolved oxygen and 80 rpm in a serum-free 302 culture medium (pH 7.0, SAFC). The culture product was collected by centrifugation. After the supernatant was filtered using 0.45 μm filter membrane (Millipore), affinity chromatography was performed according to the instruction manual of Protein A affinity column (GE) with the specific steps as follows: initially, a protein A affinity column was equilibrated by a PBS buffer (pH 7.0); subsequently, the supernatant was loaded on the column and washed again with the PBS buffer; finally, the column was eluted with a citric acid buffer (pH 3.0), and the eluent was collected and filtered by a 0.45 μm filter membrane. After virus inactivation by adding S/D (0.3% tributyl phosphate/1% Tween 80) at 24°C for 6 h, the target protein was further purified by a

molecular sieve chromatography with the following steps: first, the eluent obtained from the Protein A affinity chromatography was dialyzed in a dialysis bag against a PBS buffer; subsequently, the sample was concentrated in a 10 KD ultrafiltration cup (Millipore); the sample concentrated using the ultrafiltration cup was then loaded on a molecular sieve chromatography column Superdex 200 (GE) equilibrated by a PBS buffer, and subsequently the column was eluted with a PBS buffer and the eluting peak was collected. The purified protein was analyzed by SDS-PAGE (Fig. 3); and subsequently, the eluates containing the required expression product was combined and filtered with a 0.22 μm filter membrane (Millipore) before the protein content was determined using many methods such Lowry protein assay.

Example 5: Gradient-binding experiment of the fusion proteins

The binding capacities of the fusion proteins as constructed above to FGF-2 were detected by ELISA, similarly as in Example 3. Initially, a 96-well ELISA plate was coated by 100 μL solution containing 50 ng/mL FGF-2 (R&D Systems). Subsequently, the plate was washed in 300 μL PBST washing solution for 5 times before each coated well was blocked by 200 μL freshly prepared blocking working solution (KPL) (blocking stock solution : PBS = 1: 19) and incubated at 37°C for 1 h. After washed in 300 μL PBST washing solution for 5 times, 100 μL solutions containing various fusion proteins at different concentrations (the starting content of protein was 16000 pM, and was diluted in a ratio of 1: 3) were added and incubated at 37°C for 2 h. After washed in 300 μL PBST washing solution for 5 times, 100 μL anti-human IgG Fc-HRP secondary antibody (BETHYL) diluted with PBS in a ratio of 1: 10000 was added and incubated at 37°C for 1 h. After washed in 300 μL PBST washing solution for 5 times, the well was developed by adding 100 μL developing solution (KPL), and finally the development was stopped by adding 100 μL stopping solution (KPL) before the absorbance of the ELISA plate was read at a wavelength of 450 nm on a ELISA reader. Based on the intensity of the signal, the gradient binding capacities of

the fusion proteins to FGF-2 were determined. In the experiment procedure mentioned above, specific conditions and steps may be found in Example 3. Gradient binding of 26# fusion protein to FGF-2 was compared in Fig. 4. It can be seen that the binding capacity of 26# fusion protein to FGF-2 was dose-dependent. It has been suggested by this example that the binding capacity to FGF-2 increased with an enhanced molar concentration of 26# fusion protein, manifested by a stronger signal at a wavelength of 450 nm; while the binding capacity to FGF-2 decreased correspondingly with a gradient dilution of the molar concentration of 26# fusion protein.

Example 6: Affinity experiment of the fusion proteins

The affinity of the fusion protein to FGF-2 in a solution system was determined by an affinity experiment. Initially, a 96-well ELISA plate was coated by 100 μL solution containing 2.0 μg/mL FGF-2 capture antibody (R&D Systems). Subsequently, the plate was washed in 300 μL PBST washing solution for 5 times before each coated well was blocked by a blocking working solution (KPL) (as seen in Example 3) and incubated at 37°C for 1 h. After washed in 300 μL PBST washing solution for 5 times, previously prepared and incubated (4 °C overnight) mixture of the fusion proteins and FGF-2 as well as the standard (R&D Systems) diluted in a gradient were added, in which the specific preparation procedure was as follows: the starting concentration of 26# fusion protein was 400 pM (dissolved in PBS) and diluted in a gradient ratio of 2-fold, and the solutions of the fusion protein were 1: 1 mixed with 20 pM FGF-2 solution (dissolved in PBS), and that is, the starting final concentration of each fusion protein was 200pM, and the final concentration of FGF-2 was 10 pM in the mixture solution prepared. The plate was incubated at 37°C for 2 h and washed in 300 μL PBST washing solution for 5 times before 100 μL FGF-2 detection antibody solution (250 ng/mL) was added (R&D systems, which may specifically detect free antibodies against FGF-2). The plate was incubated at 37°C for 2 h and washed in 300 μL PBST washing solution for 5 times, and subsequently, HRP labeled streptavidin

(R&D systems) was added (diluted by PBS in 1: 200). The plate was incubated at 37°C for 2 h and washed in 300 μL PBST washing solution for 5 times before the well was developed at room temperature in a dark place for an appropriate duration (about 15-30 min) by adding 100 μL developing solution (KPL). Finally, after the development was stopped by adding 100 μL stopping solution (KPL), the absorbance of the ELISA plate was read at a wavelength of 450 nm on a ELISA reader. The relative concentration of free FGF-2 in the mixture of the fusion protein and FGF-2 was determined. The affinity between 26# fusion protein and FGF-2 in a solution system can be seen in Fig. 5. As demonstrated in this Example, 26# fusion protein had high affinity to FGF-2 in a solution system. The affinity increased with an enhanced concentration, which is manifested as a decreased amount of free FGF-2 with an enhanced concentration of the fusion protein. The affinity between 26# fusion protein and FGF-2 in a solution system can be seen in Fig. 5. As demonstrated in this Example, 26# fusion protein had affinity to FGF-2 in a solution system. The affinity increased with an enhanced concentration, which is manifested as a decreased amount of free FGF-2.

Example 7: Inhibitory test for division on human umbilical vein endothelial cell

The inhibitory ability of the fusion proteins on the division of vascular endothelial cells was examined in a division test for human umbilical vein endothelial cell (HUVEC). HUVEC cells (AllCells) were cultured to the exponential growth phase in an HUVEC complete medium (AllCells) at 37°C in an incubator supplemented with 5% CO_2. HUVEC cells were counted after digested by trypsin. 3000 HUVEC cells were inoculated per well in an HUVEC basal medium containing 1% FBS (AllCells) in a 96-well plate. The plate was cultured overnight at 37°C in an incubator supplemented with 5% CO_2.

100 μL FGF-2 (R&D Systems) solution (final concentration of 5 ng/mL) diluted by an HUVEC basal medium containing 1% FBS, as well as 100 μL mixture of

various amount of 26# fusion protein and FGF-2 (in which the final concentration of the fusion protein was 40 pM, diluted in an HUVEC basal medium containing 1% FBS with a ratio of 1:10, and the final concentration of FGF-2 was 5 ng/mL) were added and cultured for another 3-4 days. Subsequently, the culture medium was taken out and a culture medium containing 10% CCK-8 (DOJINDO) was added for another 2 h of culture before the absorbance of the 96-well plate was read directly at a wavelength of 450 nm on an ELISA reader. Based on the difference of the absorbance, the inhibitory ability of the fusion protein on the division of vascular endothelial cells induced by FGF-2 was determined. The effect of the fusion protein on HUVEC cell division induced by FGF-2 was shown in Fig. 6. As demonstrated in this Example, 26# fusion protein has biological activity and function at the cellular level, which can inhibit HUVEC cell division induced by FGF-2, and has the binding capacity to FGF-2. Such binding capacity increases as the molar concentration of 26# fusion protein increases, which is indicated by the inhibition of HUVEC cell division induced by FGF-2.

The present invention has already been illustrated by specific examples. However, it will be appreciated by a person of ordinary skill in the art that the present invention is not limited to each specific embodiments. Various changes and modifications may be made by a person of ordinary skill under the scope of the present invention, and each technical feature mentioned in the specification may be combined without departing from the spirit and scope of the invention. Such changes and modifications fall into the scope of the present invention.

References

[1] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell, 2000, 100(1):57–70.

[2] Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell. 1996, 86:353-64.

[3] Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med. 2003, 9:669-76.

[4] Ferrara N. Vascular endothelial growth factor as a target for anticancer therapy. Oncologist. 2004, 1:2-10.

[5] Jenab-Wolcott J, Giantonio BJ. Bevacizumab: current indications and future development for management of solid tumors. Expert Opin Biol Ther. 2009, 9(4):507-17.

[6] Summers J, Cohen MH, Keegan P, Pazdur R. FDA drug approval summary: bevacizumab plus interferon for advanced renal cell carcinoma. Oncologist. 2010, 15(1):104-11.

[7] Hsu JY, Wakelee HA. Monoclonal antibodies targeting vascular endothelial growth factor: current status and future challenges in cancer therapy. BioDrugs. 2009, 23(5):289-304.

[8] Krupitskaya Y, Wakelee HA. Ramucirumab, a fully human mAb to the transmembrane signaling tyrosine kinase VEGFR-2 for the potential treatment of cancer. Curr Opin Investig Drugs. 2009, 10(6):597-605.

[9] Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004, 350(23):2335-42.

[10] Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, Johnson DH. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med. 2006, 355(24):2542-50.

[11] Jenab-Wolcott J, Giantonio BJ. Bevacizumab: current indications and future development for management of solid tumors. Expert Opin Biol Ther, 2009, 9(4):507-17.

[12] Dorrell MI, Aguilar E, Scheppke L, Barnett FH, Friedlander M. Combination angiostatic therapy completely inhibits ocular and tumor angiogenesis. Proc Natl Acad Sci U S A. 2007, 104(3):967-72.

[13] Casanovas O, Hicklin DJ, Bergers G, Hanahan D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell. 2005, 8(4):299-309.

[14] A Beenken, M Mohammadi. Nature Rev., 2009, 8(3): 235~53.

[15] M Mohammadi, S K Olsen, O A Ibrahimi. Cytokine, 2005, 16: 107~137.

[16] M Presta, P D Era, S Mitola et al. Cytokine, 2005, 16(2): 159~178.

[17] R Grose, C Dickson. Cytokine, 2005, 16(2): 179~186.

[18] Y H Cao, R H Cao, E M Hedlund. J. Mol. Med., 2008, 86(7): 785~789.

[19] M J Cross, L C Welsh. Trends Pharmacol. Sci., 2001, 22(4): 201~207.

[20] Wang F. et al., J Biol Chem. 1995, 270:10231-10235

[21]Olsen SK. et al., Proc Natl Acad Sci U S A. 2004, 101:935-940

[22]Gauglhofer C, Sagmeister S, Schrottmaier W, Fischer C, Rodgarkia-Dara C, Mohr T, Stättner S, Bichler C, Kandioler D, Wrba F, Schulte-Hermann R, Holzmann K, Grusch M, Marian B, Berger W, Grasl-Kraupp B. Hepatology. 2011 Mar;53(3):854-64.

CLAIMS

1. An isolated soluble fusion protein of fibroblast growth factor receptor (FGFR), comprising: a part derived from the FGFR extracellular domain and an immunoglobulin Fc region, wherein the part derived from the FGFR extracellular domain consists of an amino acid sequence corresponding to positions 1-241 of SEQ ID NO: 12, positions 1-230 of SEQ ID NO: 13, or positions 1-224 of SEQ ID NO: 14 or encoded by a nucleotide sequence corresponding to positions 1-723 of SEQ ID NO: 19, positions 1-690 of SEQ ID NO: 20, or positions 1-672 of SEQ ID NO: 21.

2. An isolated soluble fusion protein of fibroblast growth factor receptor (FGFR), wherein said protein consists of an amino acid sequence of any one of SEQ ID NOs: 12-14, or is encoded by a nucleic acid molecule consisting of a nucleotide sequence of any one of SEQ ID NOs: 19-21.

3. An isolated nucleic acid molecule encoding the fusion protein of claim 1 or claim 2.

4. A vector comprising the nucleic acid molecule of claim 3.

5. A cell, optionally a CHO cell, transfected by the vector of claim 4.

6. A pharmaceutical composition, comprising the fusion protein of claim 1 or claim 2, the nucleic acid molecule of claim 3, the vector of claim 4, or the cell of claim 5, and a pharmaceutically acceptable carrier.

7. Use of the fusion protein of claim 1 or claim 2, the nucleic acid molecule of claim 3, the vector of claim 4, or the cell of claim 5 in the manufacture of a medicament for inhibiting angiogenesis in a mammal.

8. Use of the fusion protein of claim 1 or claim 2, the nucleic acid molecule of claim 3, the vector of claim 4, or the cell of claim 5 in the manufacture of a medicament for treating or preventing a tumor, optionally a solid tumor, in a subject.

9. A method for inhibiting angiogenesis in a mammal in need thereof, comprising administering an effective amount of the fusion protein of claim 1 or claim 2, the nucleic acid molecule of claim 3, the vector of claim 4, the cell of claim 5, or the pharmaceutical composition of claim 6 to the mammal.

10. A method for treating or preventing a tumor, optionally a solid tumor, in a subject, comprising administering an effective amount of the fusion protein of claim 1 or claim 2, the nucleic acid molecule of claim 3, the vector of claim 4, the cell of claim 5, or the pharmaceutical composition of claim 6 to the subject.

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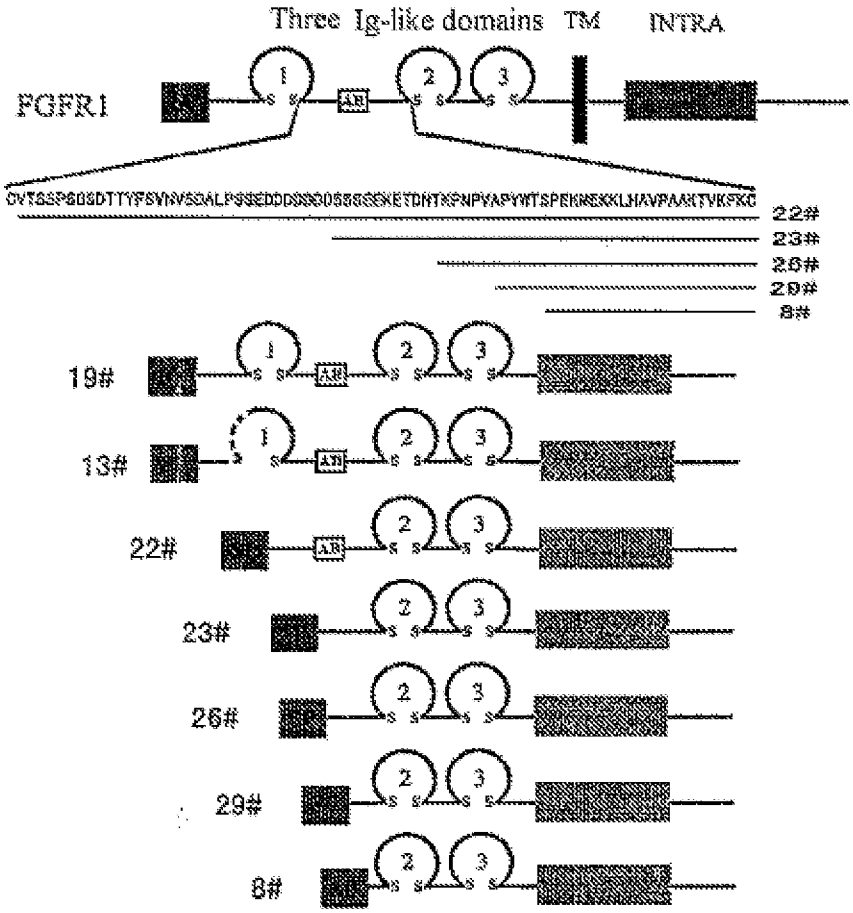
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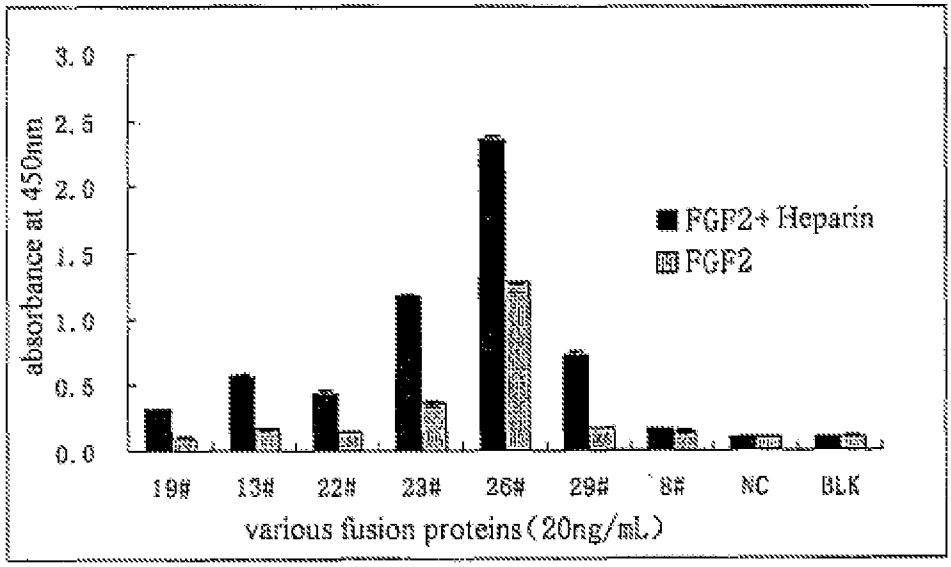
Figure 2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BLK
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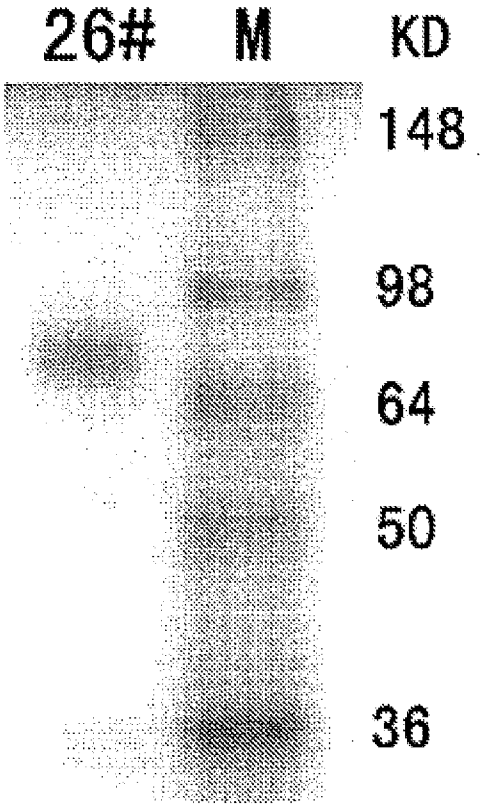
Figure 3



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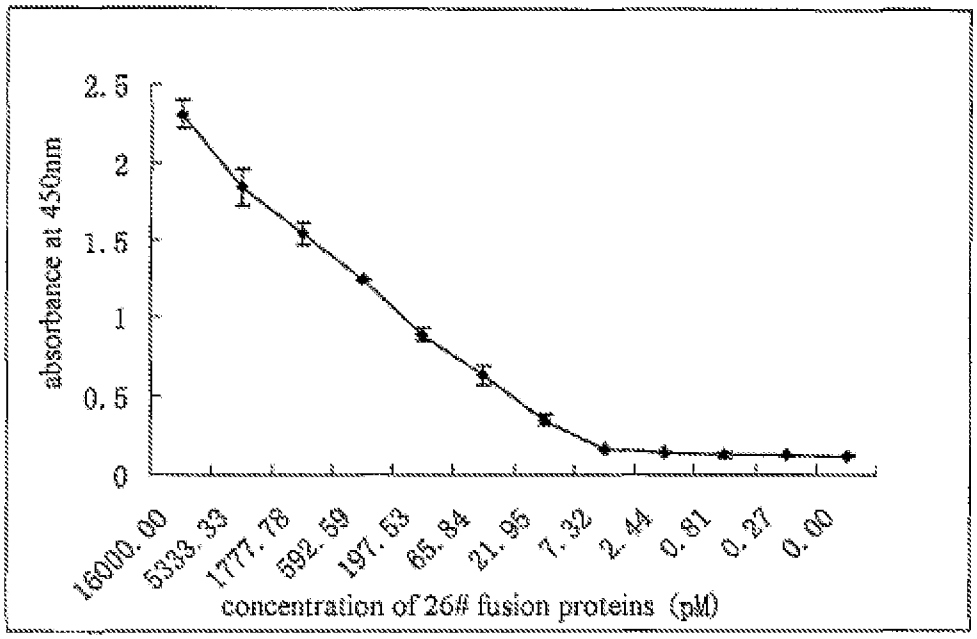

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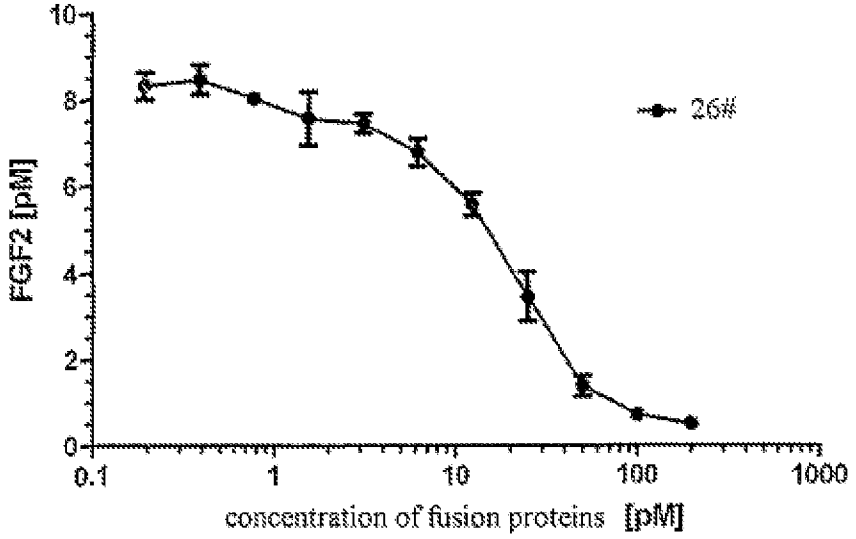

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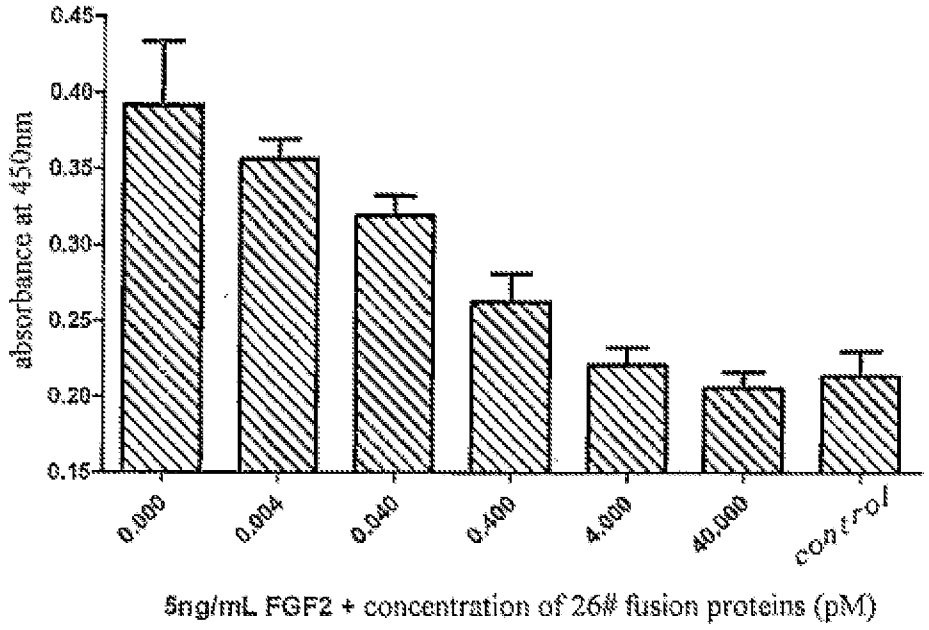
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0.30
0.25
0.20
0.15
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4.000
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control
5ng/mL FGF2 + concentration of 26# fusion proteins (pM)

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<211> 6055
<212> DNA
<213> Homo sapiens

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<210> 7
<211> 223
<212> PRT
<213> Homo sapiens

<400> 7

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Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
20 25 30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
35 40 45

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
50 55 60

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
65 70 75 80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
85 90 95

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

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
115 120 125

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
130 135 140

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
145 150 155 160

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
165 170 175

Asp Gly Pro Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
180 185 190

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
195 200 205

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
210 215 220

<210> 8
<211> 669
<212> DNA
<213> Homo sapiens

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gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg 180

cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 240

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aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 360

gaaccacagg tgtacaccct gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc 420

ctgacctgcc tggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 480

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ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 600

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ccgggtaaa 669

<210> 9
<211> 580
<212> PRT
<213> Artificial sequence

<220>
<223> 19# fused protein

<400> 9

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Val Glu Val Glu Ser Phe Leu Val His Pro Gly Asp Leu Leu Gln Leu
            20                  25                  30

Arg Cys Arg Leu Arg Asp Asp Val Gln Ser Ile Asn Trp Leu Arg Asp
        35                  40                  45

Gly Val Gln Leu Ala Glu Ser Asn Arg Thr Arg Ile Thr Gly Glu Glu
    50                  55                  60

Val Glu Val Gln Asp Ser Val Pro Ala Asp Ser Gly Leu Tyr Ala Cys
65                  70                  75                  80

Val Thr Ser Ser Pro Ser Gly Ser Asp Thr Thr Tyr Phe Ser Val Asn
                85                  90                  95

Val Ser Asp Ala Leu Pro Ser Ser Glu Asp Asp Asp Asp Asp Asp Asp
            100                 105                 110

Ser Ser Ser Glu Glu Lys Glu Thr Asp Asn Thr Lys Pro Asn Pro Val
        115                 120                 125

Ala Pro Tyr Trp Thr Ser Pro Glu Lys Met Glu Lys Lys Leu His Ala
    130                 135                 140

Val Pro Ala Ala Lys Thr Val Lys Phe Lys Cys Pro Ser Ser Gly Thr
145                 150                 155                 160

Pro Asn Pro Thr Leu Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro
                165                 170                 175

Asp His Arg Ile Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile
            180                 185                 190

Ile Met Asp Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile
        195                 200                 205

Val Glu Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr Gln Leu Asp Val
    210                 215                 220

Val Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala
225                 230                 235                 240

Asn Lys Thr Val Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val
                245                 250                 255
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Tyr Ser Asp Pro Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val
            260                 265                 270

Asn Gly Ser Lys Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu
        275                 280                 285

Lys Thr Ala Gly Val Asn Thr Thr Asp Lys Glu Met Glu Val Leu His
    290                 295                 300

Leu Arg Asn Val Ser Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala
305                 310                 315                 320

Gly Asn Ser Ile Gly Leu Ser His His Ser Ala Trp Leu Thr Val Leu
                325                 330                 335

Glu Ala Leu Glu Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Leu
            340                 345                 350

Glu Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
        355                 360                 365

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
    370                 375                 380

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
385                 390                 395                 400

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
                405                 410                 415

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
            420                 425                 430

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
        435                 440                 445

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
    450                 455                 460

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
465                 470                 475                 480

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
                485                 490                 495

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
            500                 505                 510

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
        515                 520                 525
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Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
    530                 535                 540

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
545                 550                 555                 560

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
                565                 570                 575

Ser Pro Gly Lys
            580
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<210> 10
<211> 526
<212> PRT
<213> artificial sequence

<220>
<223> 13# fused protein

<400> 10

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Val Pro Ala Asp Ser Gly Leu Tyr Ala Cys Val Thr Ser Ser Pro Ser
            20                  25                  30

Gly Ser Asp Thr Thr Tyr Phe Ser Val Asn Val Ser Asp Ala Leu Pro
        35                  40                  45

Ser Ser Glu Asp Asp Asp Asp Asp Asp Asp Ser Ser Ser Glu Glu Lys
    50                  55                  60

Glu Thr Asp Asn Thr Lys Pro Asn Pro Val Ala Pro Tyr Trp Thr Ser
65                  70                  75                  80

Pro Glu Lys Met Glu Lys Lys Leu His Ala Val Pro Ala Ala Lys Thr
                85                  90                  95

Val Lys Phe Lys Cys Pro Ser Ser Gly Thr Pro Asn Pro Thr Leu Arg
            100                 105                 110

Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro Asp His Arg Ile Gly Gly
        115                 120                 125

Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile Ile Met Asp Ser Val Val
    130                 135                 140

Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile Val Glu Asn Glu Tyr Gly
145                 150                 155                 160

Ser Ile Asn His Thr Tyr Gln Leu Asp Val Val Glu Arg Ser Pro His
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165 170 175

Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Lys Thr Val Ala Leu
180 185 190

Gly Ser Asn Val Glu Phe Met Cys Lys Val Tyr Ser Asp Pro Gln Pro
195 200 205

His Ile Gln Trp Leu Lys His Ile Glu Val Asn Gly Ser Lys Ile Gly
210 215 220

Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu Lys Thr Ala Gly Val Asn
225 230 235 240

Thr Thr Asp Lys Glu Met Glu Val Leu His Leu Arg Asn Val Ser Phe
245 250 255

Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn Ser Ile Gly Leu
260 265 270

Ser His His Ser Ala Trp Leu Thr Val Leu Glu Ala Leu Glu Glu Arg
275 280 285

Pro Ala Val Met Thr Ser Pro Leu Tyr Leu Glu Asp Lys Thr His Thr
290 295 300

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
305 310 315 320

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
325 330 335

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
340 345 350

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
355 360 365

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
370 375 380

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
385 390 395 400

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
405 410 415

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
420 425 430

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val

435 440 445

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
450 455 460

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
465 470 475 480

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
485 490 495

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
500 505 510

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
515 520 525

<210> 11
<211> 500
<212> PRT
<213> artificial sequence

<220>
<223> 22# fused protein

<400> 11

Val Thr Ser Ser Pro Ser Gly Ser Asp Thr Thr Tyr Phe Ser Val Asn
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Val Ser Asp Ala Leu Pro Ser Ser Glu Asp Asp Asp Asp Asp Asp Asp
20 25 30

Ser Ser Ser Glu Glu Lys Glu Thr Asp Asn Thr Lys Pro Asn Pro Val
35 40 45

Ala Pro Tyr Trp Thr Ser Pro Glu Lys Met Glu Lys Lys Leu His Ala
50 55 60

Val Pro Ala Ala Lys Thr Val Lys Phe Lys Cys Pro Ser Ser Gly Thr
65 70 75 80

Pro Asn Pro Thr Leu Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro
85 90 95

Asp His Arg Ile Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile
100 105 110

Ile Met Asp Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile
115 120 125

Val Glu Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr Gln Leu Asp Val
130 135 140

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Val Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala
145                 150                 155                 160

Asn Lys Thr Val Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val
                165                 170                 175

Tyr Ser Asp Pro Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val
            180                 185                 190

Asn Gly Ser Lys Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu
        195                 200                 205

Lys Thr Ala Gly Val Asn Thr Thr Asp Lys Glu Met Glu Val Leu His
    210                 215                 220

Leu Arg Asn Val Ser Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala
225                 230                 235                 240

Gly Asn Ser Ile Gly Leu Ser His His Ser Ala Trp Leu Thr Val Leu
                245                 250                 255

Glu Ala Leu Glu Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Leu
            260                 265                 270

Glu Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
        275                 280                 285

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
    290                 295                 300

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
305                 310                 315                 320

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
                325                 330                 335

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
            340                 345                 350

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
        355                 360                 365

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
    370                 375                 380

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
385                 390                 395                 400

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
                405                 410                 415
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Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
            420                 425                 430

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
        435                 440                 445

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
    450                 455                 460

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
465                 470                 475                 480

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
                485                 490                 495

Ser Pro Gly Lys
            500
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<210> 12
<211> 468
<212> PRT
<213> artificial sequence

<220>
<223> 23# fused protein

<400> 12

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Ala Pro Tyr Trp Thr Ser Pro Glu Lys Met Glu Lys Lys Leu His Ala
            20                  25                  30

Val Pro Ala Ala Lys Thr Val Lys Phe Lys Cys Pro Ser Ser Gly Thr
        35                  40                  45

Pro Asn Pro Thr Leu Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro
    50                  55                  60

Asp His Arg Ile Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile
65                  70                  75                  80

Ile Met Asp Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile
                85                  90                  95

Val Glu Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr Gln Leu Asp Val
            100                 105                 110

Val Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala
        115                 120                 125

Asn Lys Thr Val Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val
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130 135 140

Tyr Ser Asp Pro Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val
145 150 155 160

Asn Gly Ser Lys Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu
165 170 175

Lys Thr Ala Gly Val Asn Thr Thr Asp Lys Glu Met Glu Val Leu His
180 185 190

Leu Arg Asn Val Ser Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala
195 200 205

Gly Asn Ser Ile Gly Leu Ser His His Ser Ala Trp Leu Thr Val Leu
210 215 220

Glu Ala Leu Glu Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Leu
225 230 235 240

Glu Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
245 250 255

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
260 265 270

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
275 280 285

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
290 295 300

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
305 310 315 320

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
325 330 335

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
340 345 350

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
355 360 365

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
370 375 380

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
385 390 395 400

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro

405 410 415

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
420 425 430

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
435 440 445

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
450 455 460

Ser Pro Gly Lys
465

<210> 13
<211> 457
<212> PRT
<213> artificial sequence

<220>
<223> 26# fused protein

<400> 13

Lys Pro Asn Pro Val Ala Pro Tyr Trp Thr Ser Pro Glu Lys Met Glu
1 5 10 15

Lys Lys Leu His Ala Val Pro Ala Ala Lys Thr Val Lys Phe Lys Cys
20 25 30

Pro Ser Ser Gly Thr Pro Asn Pro Thr Leu Arg Trp Leu Lys Asn Gly
35 40 45

Lys Glu Phe Lys Pro Asp His Arg Ile Gly Gly Tyr Lys Val Arg Tyr
50 55 60

Ala Thr Trp Ser Ile Ile Met Asp Ser Val Val Pro Ser Asp Lys Gly
65 70 75 80

Asn Tyr Thr Cys Ile Val Glu Asn Glu Tyr Gly Ser Ile Asn His Thr
85 90 95

Tyr Gln Leu Asp Val Val Glu Arg Ser Pro His Arg Pro Ile Leu Gln
100 105 110

Ala Gly Leu Pro Ala Asn Lys Thr Val Ala Leu Gly Ser Asn Val Glu
115 120 125

Phe Met Cys Lys Val Tyr Ser Asp Pro Gln Pro His Ile Gln Trp Leu
130 135 140

Lys His Ile Glu Val Asn Gly Ser Lys Ile Gly Pro Asp Asn Leu Pro
145 150 155 160

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Tyr Val Gln Ile Leu Lys Thr Ala Gly Val Asn Thr Thr Asp Lys Glu
                165                 170                 175

Met Glu Val Leu His Leu Arg Asn Val Ser Phe Glu Asp Ala Gly Glu
            180                 185                 190

Tyr Thr Cys Leu Ala Gly Asn Ser Ile Gly Leu Ser His His Ser Ala
        195                 200                 205

Trp Leu Thr Val Leu Glu Ala Leu Glu Glu Arg Pro Ala Val Met Thr
    210                 215                 220

Ser Pro Leu Tyr Leu Glu Asp Lys Thr His Thr Cys Pro Pro Cys Pro
225                 230                 235                 240

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
                245                 250                 255

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            260                 265                 270

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
        275                 280                 285

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    290                 295                 300

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
305                 310                 315                 320

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                325                 330                 335

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
            340                 345                 350

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
        355                 360                 365

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    370                 375                 380

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
385                 390                 395                 400

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                405                 410                 415

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
            420                 425                 430
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Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
435 440 445

Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 14
<211> 451
<212> PRT
<213> artificial sequence

<220>
<223> 29# fused protein

<400> 14

Pro Tyr Trp Thr Ser Pro Glu Lys Met Glu Lys Lys Leu His Ala Val
1 5 10 15

Pro Ala Ala Lys Thr Val Lys Phe Lys Cys Pro Ser Ser Gly Thr Pro
20 25 30

Asn Pro Thr Leu Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro Asp
35 40 45

His Arg Ile Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile Ile
50 55 60

Met Asp Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile Val
65 70 75 80

Glu Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr Gln Leu Asp Val Val
85 90 95

Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
100 105 110

Lys Thr Val Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val Tyr
115 120 125

Ser Asp Pro Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val Asn
130 135 140

Gly Ser Lys Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu Lys
145 150 155 160

Thr Ala Gly Val Asn Thr Thr Asp Lys Glu Met Glu Val Leu His Leu
165 170 175

Arg Asn Val Ser Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly
180 185 190

Asn Ser Ile Gly Leu Ser His His Ser Ala Trp Leu Thr Val Leu Glu

195 200 205

Ala Leu Glu Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Leu Glu
210 215 220

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

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
355 360 365

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Lys
450

<210> 15

<211> 446
<212> PRT
<213> artificial sequence

<220>
<223> 8# fused protein

<400> 15

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Pro Glu Lys Met Glu Lys Lys Leu His Ala Val Pro Ala Ala Lys Thr
1               5                  10                  15

Val Lys Phe Lys Cys Pro Ser Ser Gly Thr Pro Asn Pro Thr Leu Arg
            20                  25                  30

Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro Asp His Arg Ile Gly Gly
        35                  40                  45

Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile Ile Met Asp Ser Val Val
    50                  55                  60

Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile Val Glu Asn Glu Tyr Gly
65                  70                  75                  80

Ser Ile Asn His Thr Tyr Gln Leu Asp Val Val Glu Arg Ser Pro His
                85                  90                  95

Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Lys Thr Val Ala Leu
            100                 105                 110

Gly Ser Asn Val Glu Phe Met Cys Lys Val Tyr Ser Asp Pro Gln Pro
        115                 120                 125

His Ile Gln Trp Leu Lys His Ile Glu Val Asn Gly Ser Lys Ile Gly
    130                 135                 140

Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu Lys Thr Ala Gly Val Asn
145                 150                 155                 160

Thr Thr Asp Lys Glu Met Glu Val Leu His Leu Arg Asn Val Ser Phe
                165                 170                 175

Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn Ser Ile Gly Leu
            180                 185                 190

Ser His His Ser Ala Trp Leu Thr Val Leu Glu Ala Leu Glu Glu Arg
        195                 200                 205

Pro Ala Val Met Thr Ser Pro Leu Tyr Leu Glu Asp Lys Thr His Thr
    210                 215                 220

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
225                 230                 235                 240
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Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
245 250 255

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
260 265 270

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
275 280 285

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
290 295 300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305 310 315 320

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
325 330 335

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
340 345 350

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
355 360 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
370 375 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
385 390 395 400

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
405 410 415

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
420 425 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 16
<211> 1743
<212> DNA
<213> Artificial sequence

<220>
<223> Nucleotide sequence of 19# fused protein

<400> 16
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tccttcctgg tccaccccgg tgacctgcta cagcttcgct gtcggctgcg ggacgatgtg 120

cagagcatca actggctgcg ggacggggtg cagctggcgg aaagcaatcg cacccgcatc 180

acagggagg aggtggaggt gcaggactcc gtgcccgcag actccggcct ctatgcttgc 240

gtaaccagca gcccctcggg cagtgacacc acctacttct ccgtcaatgt ttcagatgct 300

ctcccctcct cggaggatga tgatgatgat gatgactcct cttcagagga gaaagaaaca 360

gataacacca aaccaaaccc cgtagctcca tattggacat ccccagaaaa gatggaaaag 420

aaattgcatg cagtgccggc tgccaagaca gtgaagttca aatgcccttc cagtgggacc 480

ccaaacccca cactgcgctg gttgaaaaat ggcaaagaat tcaaacctga ccacagaatt 540

ggaggctaca aggtccgtta tgccacctgg agcatcataa tggactctgt ggtgccctct 600

gacaagggca actacacctg cattgtggag aatgagtacg gcagcatcaa ccacacatac 660

cagctggatg tcgtggagcg gtcccctcac cggcccatcc tgcaagcagg gttgcccgcc 720

aacaaaacag tggccctggg tagcaacgtg gagttcatgt gtaaggtgta cagtgacccg 780

cagccgcaca tccagtggct aaagcacatc gaggtgaatg ggagcaagat tggcccagac 840

aacctgcctt atgtccagat cttgaagact gctggagtta ataccaccga caaagagatg 900

gaggtgcttc acttaagaaa tgtctccttt gaggacgcag gggagtatac gtgcttggcg 960

ggtaactcta tcggactctc ccatcactct gcatggttga ccgttctgga agccctggaa 1020

gagaggccgg cagtgatgac ctcgcccctg tacctggagg acaaaactca cacatgccca 1080

ccgtgcccag cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc 1140

aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc 1200

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 1260

aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 1320

gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc 1380

ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 1440

gtgtacaccc tgccccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc 1500

ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 1560

gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctac 1620

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1680

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tga 1743

<210> 17
<211> 1581
<212> DNA
<213> Artificial sequence

<220>
<223> Nucleotide sequence of 13# fused protein

<400> 17
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tccggcctct atgcttgcgt aaccagcagc ccctcgggca gtgacaccac ctacttctcc 120

gtcaatgttt cagatgctct cccctcctcg gaggatgatg atgatgatga tgactcctct 180

tcagaggaga aagaaacaga taacaccaaa ccaaacccag tagctccata ttggacatcc 240

ccagaaaaga tggaaaagaa attgcatgca gtgccggctg ccaagacagt gaagttcaaa 300

tgcccttcca gtgggacccc aaaccccaca ctgcgctggt tgaaaaatgg caaagaattc 360

aaacctgacc acagaattgg aggctacaag gtccgttatg ccacctggag catcataatg 420

gactctgtgg tgccctctga caagggcaac tacacctgca ttgtggagaa tgagtacggc 480

agcatcaacc acacatacca gctggatgtc gtggagcggt cccctcaccg gcccatcctg 540

caagcagggt tgcccgccaa caaaacagtg gccctgggta gcaacgtgga gttcatgtgt 600

aaggtgtaca gtgacccgca gccgcacatc cagtggctaa agcacatcga ggtgaatggg 660

agcaagattg gcccagacaa cctgccttat gtccagatct tgaagactgc tggagttaat 720

accaccgaca aagagatgga ggtgcttcac ttaagaaatg tctcctttga ggacgcaggg 780

gagtatacgt gcttggcggg taactctatc ggactctccc atcactctgc atggttgacc 840

gttctggaag ccctggaaga gaggccggca gtgatgacct cgcccctgta cctggaggac 900

aaaactcaca catgcccacc gtgcccagca cctgaactcc tgggggacc gtcagtcttc 960

ctcttccccc caaaacccaa ggacaccctc atgatctccc ggacccctga ggtcacatgc 1020

gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc 1080

gtggaggtgc ataatgccaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt 1140

gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc 1200

aaggtctcca acaaagccct cccagccccc atcgagaaaa ccatctccaa agccaaaggg 1260

cagccccgag aaccacaggt gtacaccctg ccccatccc gggatgagct gaccaagaac 1320

caggtcagcc tgacctgcct ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg 1380

gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac 1440

ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac 1500

gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 1560

tccctgtctc cgggtaaatg a 1581

<210> 18
<211> 1503
<212> DNA
<213> Artificial sequence

<220>
<223> Nucleotide sequence of 22# fused protein

<400> 18
gtaaccagca gccccctcggg cagtgacacc acctacttct ccgtcaatgt ttcagatgct 60

ctcccctcct cggaggatga tgatgatgat gatgactcct cttcagagga gaaagaaaca 120

gataacacca aaccaaaccc cgtagctcca tattggacat ccccagaaaa gatggaaaag 180

aaattgcatg cagtgccggc tgccaagaca gtgaagttca aatgcccttc cagtgggacc 240

ccaaacccca cactgcgctg gttgaaaaat ggcaaagaat tcaaacctga ccacagaatt 300

ggaggctaca aggtccgtta tgccacctgg agcatcataa tggactctgt ggtgccctct 360

gacaagggca actacacctg cattgtggag aatgagtacg gcagcatcaa ccacacatac 420

cagctggatg tcgtggagcg gtcccctcac cggcccatcc tgcaagcagg gttgcccgcc 480

aacaaaacag tggccctggg tagcaacgtg gagttcatgt gtaaggtgta cagtgacccg 540

cagccgcaca tccagtggct aaagcacatc gaggtgaatg ggagcaagat tggcccagac 600

aacctgcctt atgtccagat cttgaagact gctggagtta ataccaccga caaagagatg 660

gaggtgcttc acttaagaaa tgtctccttt gaggacgcag gggagtatac gtgcttggcg 720

ggtaactcta tcggactctc ccatcactct gcatggttga ccgttctgga agccctggaa 780

gagaggccgg cagtgatgac ctcgcccctg tacctggagg acaaaactca cacatgccca 840

ccgtgcccag cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc 900

aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc 960

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gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc 1140

ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 1200

gtgtacaccc tgccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc 1260

ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 1320

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tga 1503

<210> 19
<211> 1407
<212> DNA
<213> Artificial sequence

<220>
<223> Nucleotide sequence of 23# fused protein

<400> 19
tcctcttcag aggagaaaga aacagataac accaaaccaa acccctagc tccatattgg 60

acatccccag aaaagatgga aaagaaattg catgcagtgc cggctgccaa gacagtgaag 120

ttcaaatgcc cttccagtgg gaccccaaac cccacactgc gctggttgaa aaatggcaaa 180

gaattcaaac ctgaccacag aattggaggc tacaaggtcc gttatgccac ctggagcatc 240

ataatggact ctgtggtgcc ctctgacaag ggcaactaca cctgcattgt ggagaatgag 300

tacggcagca tcaaccacac ataccagctg gatgtcgtgg agcggtcccc tcaccggccc 360

atcctgcaag cagggttgcc cgccaacaaa acagtggccc tgggtagcaa cgtggagttc 420

atgtgtaagg tgtacagtga cccgcagccg cacatccagt ggctaaagca catcgaggtg 480

aatgggagca agattggccc agacaacctg ccttatgtcc agatcttgaa gactgctgga 540

gttaatacca ccgacaaaga gatggaggtg cttcacttaa gaaatgtctc ctttgaggac 600

gcaggggagt atacgtgctt ggcgggtaac tctatcggac tctcccatca ctctgcatgg 660

ttgaccgttc tggaagccct ggaagagagg ccggcagtga tgacctcgcc cctgtacctg 720

gaggacaaaa ctcacacatg cccaccgtgc ccagcacctg aactcctggg gggaccgtca 780

gtcttcctct tccccccaaa acccaaggac accctcatga tctcccggac ccctgaggtc 840

acatgcgtgg tggtggacgt gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg 900

gacggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg 960

taccgtgtgg tcagcgtcct caccgtcctg caccaggact ggctgaatgg caaggagtac 1020

aagtgcaagg tctccaacaa agccctccca gcccccatcg agaaaaccat ctccaaagcc 1080

aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga tgagctgacc 1140

aagaaccagg tcagcctgac ctgcctggtc aaaggcttct atcccagcga catcgccgtg 1200

gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc cgtgctggac 1260

tccgacggct ccttcttcct ctacagcaag ctcaccgtgg acaagagcag gtggcagcag 1320

gggaacgtct tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacgcagaag 1380

agcctctccc tgtctccggg taaatga 1407

<210> 20
<211> 1374
<212> DNA
<213> artificial sequence

<220>
<223> Nucleotide sequence of 26# fused protein

<400> 20
aaacctaacc ccgtagctcc atattggaca tccccagaaa agatggaaaa gaaattgcat 60

gcagtgccgg ctgccaagac agtgaagttc aaatgcccct ccagtgggac cccaaacccc 120

acactgcgct ggttgaaaaa tggcaaagaa ttcaaacctg accacagaat tggaggctac 180

aaggtccgtt atgccacctg gagcatcata atggactctg tggtgccctc tgacaagggc 240

aactacacct gcattgtgga gaatgagtac ggcagcatca accacacata ccagctggat 300

gtcgtggagc ggtcccctca ccggcccatc ctgcaagcag ggttgcccgc caacaaaaca 360

gtggccctgg gtagcaacgt ggagttcatg tgtaaggtgt acagtgaccc gcagccgcac 420

atccagtggc taaagcacat cgaggtgaat gggagcaaga ttggcccaga caacctgcct 480

tatgtccaga tcttgaagac tgctggagtt aataccaccg acaaagagat ggaggtgctt 540

cacttaagaa atgtctcctt tgaggacgca ggggagtata cgtgcttggc gggtaactct 600

atcggactct cccatcactc tgcatggttg accgttctgg aagccctgga agaggccg 660

gcagtgatga cctcgccccc gtacctggag gacaaaactc acacatgccc accgtgccca 720

gcacctgaac tcctgggggg accgtcagtc ttcctcttcc ccccaaaacc caaggacacc 780

ctcatgatct cccggacccc tgaggtcaca tgcgtggtgg tggacgtgag ccacgaagac 840

cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag 900

ccgcgggagg agcagtacaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac 960

caggactggc tgaatggcaa ggagtacaag tgcaaggtct ccaacaaagc cctcccagcc 1020

cccatcgaga aaaccatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc 1080

ctgccccccat cccgggatga gctgaccaag aaccaggtca gcctgacctg cctggtcaaa 1140

ggcttctatc ccagcgacat cgccgtggag tgggagagca atgggcagcc ggagaacaac 1200

tacaagacca cgcctcccgt gctggactcc gacggctcct tcttcctcta cagcaagctc 1260

accgtggaca agagcaggtg gcagcagggg aacgtcttct catgctccgt gatgcatgag 1320

gctctgcaca accactacac gcagaagagc ctctccctgt ctccgggtaa atga 1374

<210> 21
<211> 1356
<212> DNA
<213> Artificial sequence

<220>
<223> nucleotide sequence of 29# fused protein

<400> 21
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acagtgaagt tcaaatgccc ttccagtggg accccaaacc ccacactgcg ctggttgaaa 120

aatggcaaag aattcaaacc tgaccacaga attggaggct acaaggtccg ttatgccacc 180

tggagcatca taatggactc tgtggtgccc tctgacaagg gcaactacac ctgcattgtg 240

gagaatgagt acggcagcat caaccacaca taccagctgg atgtcgtgga gcggtccct 300

caccggccca tcctgcaagc agggttgccc gccaacaaaa cagtggccct gggtagcaac 360

gtggagttca tgtgtaaggt gtacagtgac ccgcagccgc acatccagtg gctaaagcac 420

atcgaggtga atgggagcaa gattggccca gacaacctgc cttatgtcca gatcttgaag 480

actgctggag ttaataccac cgacaaagag atggaggtgc ttcacttaag aaatgtctcc 540

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