DOWNLOAD PDF CLONING AND CHARACTERIZATION OF HFGL2, THE HUMAN COUNTERPART TO THE MOUSE GENE FGL2

Chapter 1 : Methods of modulating immune coagulation - Transplantation Technologies Inc.

In the present studies, we report the cloning and structural characterization of the HFGL2 gene and its functional role in human fulminant hepatitis. The HFGL2 gene is approximately 7 kb in length with 2 exons.

A method of inhibiting or suppressing an immune response to a porcine organ or tissue comprising administering an effective amount of an agent that inhibits porcine fql2 to the porcine organ or tissue, porcine donor or transplant recipient. A method of inhibiting or preventing thrombosis associated with xenotransplant rejection of a porcine organ or tissue comprising administering an effective amount of an agent that inhibits porcine fgl2 to the porcine organ or tissue, porcine donor or transplant recipient. A method according to claim 1 wherein the agent is an antibody that binds to porcine fgl2. A method according to claim 1 wherein the agent is an antisense oligonucleotide that is complementary to the porcine fgl2 sequence. A method according to claim 1 wherein the agent inhibits the porcine fgl2 having a nucleic acid sequence shown in FIG. A method according to claim 1 wherein the organ or tissue is from a transgenic pig lacking expression of the porcine fgl2 gene. A method of modulating an immune response comprising administering an effective amount of a porcine fgl2 nucleic acid sequence, a porcine fgl2 protein or a porcine fgl2 modulator to an animal in need thereof. A method according to claim 7 to modulate an immune response involved in graft rejection. A method according to claim 7 to modulate an immune response involved in fetal loss. A method according to claim 7 to modulate an immune response involved in a viral infection. A method according to claim 7 to modulate an immune response involved in a hepatitis-like disease. A method according to claim 7 wherein the porcine fgl2 has the nucleic acid seqeuence shown in FIG. A method according to claim 7 wherein the porcine fgl2 modulator is an antibody that binds to fgl2. A method according to claim 7 wherein the porcine fgl2 modulator is an antisense oligonucleotide that is complementary to the porcine fgl2 sequence. An isolated porcine fgl2 nucleic acid molecule having a nucleic acid sequence shown in FIG. An isolated porcine fgl2 nucleic acid molecule according to claim 15 wherein the nucleic acid sequence comprises: An isolated porcine fgl2 protein having an amino acid sequence shown in FIG. An antibody that binds to an isolated protein according to claim An antisense oligonucleotide that is complementary to the porcine fgl2 sequence of claim Despite increasing demand, low rates of donation have resulted in a chronic shortage of available organs 1. Xenotransplantation offers one potential solution to this problem. The pig has been identified as the most suitable donor of organs for use in humans for anatomical, physiological, and ethical reasons 2. Pig organs that are transplanted into nonhuman primates are destroyed by hyperacute rejection HAR within minutes to hours. HAR occurs due to the binding of preformed xenoreactive antibodies XNA to the graft endothelium, resulting in complement activation, endothelial damage, interstitial hemorrhage, thrombosis, and graft loss 3. HAR has been overcome through the use of strategies aimed at inactivating or depleting XNA and complement 5,6. One of the most promising strategies has been the use of transgenic donor pigs that express human complement regulatory molecules such as decay accelerating factor hDAF 7. Despite using organs from transgenic pigs in combination with antibody depletion and profound immune suppression, indefinite survival of pig-to-primate solid organ xenografts has not been achieved. Xenografts are lost after days to weeks due to a poorly understood process known as delayed xenograft rejection DXR 8. DXR has been associated with clinically evident abnormalities in coagulation in preclinical studies of pig-to-primate solid organ xenotransplantation 9. Thrombosis and microangiopathy are the major pathological features observed in rejected grafts; cellular infiltration is a less prominent feature. Mounting evidence suggests that complement components, coagulation factors, thromboregulatory pathways, leukocytes, cytokines, and antibodies may all play important roles in the pathogenesis of DXR Role of the endothelium in xenograft thrombosis. Elucidating the processes that contribute to thrombosis after xenotransplantation is, therefore, a principal focus of ongoing research efforts. The vascular endothelium is critically involved in regulating coagulation, and is the initial site of interaction between the xenograft and the recipient. Activation of endothelial cells EC in the context of xenograft

rejection has been identified both in vivo and in vitro Events that occur include acute changes in cell morphology, such as shape change and retraction; release of substances such as heparan sulfate, von Willebrand factor, and tissue plasminogen activator; surface expression of molecules such as P-selectin and platelet activating factor; and loss of anticoagulant proteins such as thrombomodulin VCAM-1 , proinflammatory cytokines eg. IL-1 , and procoagulant molecules eg. A wide range of xenogeneic stimuli have been observed to result in Type II activation of porcine vascular endothelial cells. Activated endothelial cells may directly contribute to the generation of a prothrombotic state within a solid organ xenograft. After activation by NK cells in vitro, porcine EC demonstrate an induction of procoagulant function More specifically, two groups have independently demonstrated that cultured porcine aortic endothelial cells PAEC directly cleave human prothrombin to thrombin by an unidentified mechanism, in the absence of the classical prothrombinase complex 27, The production of thrombin, a central mediator of coagulation and inflammation, appears to play an important role in xenotransplantation; in vivo inhibition of thrombin is associated with prolonged xenograft survival 29, Fibrinogen-like protein 2 fgl2. Fgl2 is a novel procoagulant molecule that possesses direct prothrombinase activity. It has been implicated in the thrombosis associated with viral hepatitis, fetal loss syndromes, and transplant rejection. Fgl2 was initially described as a cytokine-induced procoagulant activity PCA in murine lymphoid cells, which were demonstrated to activate prothrombin directly to thrombin in the absence of factor VII or factor X Subsequently, fulminant hepatic failure induced by murine hepatitis virus strain 3 MHV-3 infection in susceptible mice was shown to be associated with a marked rise in monocyte PCA 32 ; monoclonal antibodies generated against PCA were shown to prevent mortality in these mice Using these antibodies, a novel murine procoagulant was functionally cloned by screening of murine peritoneal macrophage cDNA libraries synthesized from MHV-3 infected mice 34, Sequence analysis of the MHV-3 induced prothrombinase revealed homology to musfiblp, a previously described gene encoding a mouse fibrinogen-like protein. The mRNA transcript encoding the human homologue of this molecule was subsequently isolated from T-lymphocytes, and was termed fibroleukin due to its homology with fibrinogen 38, The human gene encoding fibroleukin hfgl2 , was recently cloned and characterized, and studies have suggested a role for the molecule in the pathogenesis of fulminant viral hepatitis in humans 40, Recent experiments have also identified a principal role for fgl2 in rodent models of spontaneous abortion Fgl2 is highly conserved between mice and humans. The murine fgl2 mfgl2 and hfgl2 genes localize to synthetic chromosomal loci on chromosomes 5 and 7, respectively. Comprised of two exons, both genes encode two mRNA transcripts of approximately 1. The longest open reading frame encodes a protein of amino acids in mice, and amino acids in humans. The constitutive function of fgl2 is not well understood, as the molecule has been predominantly studied in its role as an induced procoagulant. Recent experiments in our laboratory suggest that fgl2 is a membrane-bound serine protease that independently cleaves prothrombin to thrombin. Site-directed mutagenesis of serine residue 89 to alanine abolishes the prothrombinase activity. Additional experiments suggest that fgl2 may have an immunoregulatory function. Several experiments suggest that fgl2 is implicated in allograft rejection. Elevations in factor VII independent monocyte procoagulant activity PCA have been shown to be associated with renal allograft rejection in humans 45, An increase in PCA has also been observed to correlate with small intestinal allograft rejection in rodent models 47, Rejection of heterotopic murine cardiac allografts has been associated with increased fgl2 expression in graft endothelial cells and infiltrating leukocytes Wild-type mouse hearts transplanted heterotopically into rats develop intravascular thrombosis and other typical features of xenograft rejection in association with increased tissue levels of fgl2 mRNA. The use of donor hearts from fgl2 knockout mice dramatically reduces the amount of thrombosis observed In view of the foregoing, there is a need in the art to clone and characterize the porcine fgl2 as a potential target for genetic modification in the pig, in order to prevent thrombosis and rejection of pig-to-primate solid organ xenografts. Modulation of fgl2 expression in porcine organs or tissues that are transplanted into humans or nonhuman primates will ameliorate the thrombosis that is currently seen with pig-to-primate solid organ xenotransplantation. Accordingly, in one

aspect, the present invention provides an isolated porcine fgl2 molecule or a homolog or analog thereof. In one embodiment, the present invention provides an isolated porcine fgl2 molecule having the nucleic acid shown in FIG. In another embodiment, the present invention provides an isolated porcine fgl2 molecule having the amino acid sequence found in FIG. The present invention also includes agonists and antagonists of porcine fgl2 function or activity including antisense molecules and antibodies to porcine fgl2. The present invention includes a method of immune modulation comprising administering an effective amount of a porcine fgl2 nucleic acid or protein or an agonist or antogonist thereof to a cell or animal in need thereof. In one aspect, the present invention provides a method of modulating an immune response by administering an effective amount of an agent that inhibits the activity of porcine fgl2. An agent that inhibits the interaction of the porcine fgl2 protein may be an antibody that binds to the porcine fgl2 protein. Accordingly, the invention includes a method of immune modulation comprising administering an effective amount of an antibody that binds to a porcine fgl2 molecule to a cell or animal in need thereof. In one embodiment of the invention, the immune modulation is immune suppression. Such methods of immune suppression may be useful in preventing the prothrombinase activity of porcine fgl2 which would be useful when transplanting pig organs to other animals. Accordingly, in one embodiment the present invention provides a method of preventing thrombosis associated with xenotransplant rejection of a porcine organ or tissue comprising administering an effective amount of an agent that inhibits the activity of porcine fgl2 to the porcine organ or tissue or donor. In one embodiment, the agent is an antibody that inhibits the activity of porcine fgl2. In another embodiment, the agent is an antisense molecule of the porcine fgl2 nucleic acid sequence. In yet another aspect, the present invention includes screening methods for identifying substances which are capable of binding to the porcine fgl2 molecules described herein. In particular, the methods may be used to identify substances or agonists which are capable of binding to and augmenting or attenuating the effects of porcine fgl2. Alternatively, the methods may be used to identify substances or antagonists which are capable of binding to porcine fgl2 and which inhibit the effects or activity of porcine fgl2. Accordingly, the invention provides a method of identifying substances which bind with a porcine fgl2 protein, comprising the steps of: Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description. FISH signals are localized to one porcine chromosome arrow, left. Staining of the same mitotic figure with DAPI demonstrates that the signals are localized to chromosome 9 right. Panel B is a schematic showing the localization of the porcine fgl2 gene to porcine chromosome 9, region q16q Each dot represents one pair of FISH signals detected from one out of ten images analyzed. Next two panels panels B and C show effect of withdrawing immunosuppression on day 60 with marked cellular rejection looking like allo rejection not xeno rejection. No evidence of vascular thrombosis or hemorrhage FIG. Porcine fgl2 As hereinbefore mentioned, the present inventors have isolated, cloned and sequenced the porcine fgl2 gene. Accordingly, the present invention provides an isolated porcine fgl2 having a nucleic acid sequence shown in FIG. The present invention also provides an isolated porcine fgl2 having a nucleic acid sequence that encodes an fgl2 protein having an amino acid sequence shown in FIG. The term also includes modified or substituted sequences comprising non-naturally occurring monomers or portions thereof, which function similarly. The nucleic acid sequences of the present invention may be ribonucleic RNA or deoxyribonucleic acids DNA and may contain naturally occurring bases including adenine, guanine, cytosine, thymidine and uracil.

Chapter 2 : USB2 - FGL-2 prothrombinase as a diagnostic tool for malignancy - Google Patents

the pivotal role played by a direct prothrombinase Idce activity, encoded by the gene fgi2, in the pathogenesis of MHV-3 induced fulminant hepatic fÃ ilure. This thesis reports the cloning and stnictural characterization of the human counterpart to fgi2, namely hfgi2, fiom the PAC genomic library.

Thus, the present invention provides FGL-2 prothrombinase activity as a diagnostic tool for malignancy. The human gene is approximately 7 kb in length with 2 exons. From the nucleotide sequence of the human gene a amino acid long protein is predicted. FGL-2 prothrombinase is a transmembrane protein which was shown to have a serine protease activity capable of directly cleaving prothrombin to thrombin in the absence of factor VII or factor X leading to fibrin deposition, thus triggering thrombosis [Ning et al. Moreover, it was shown that distinct domains of FGL-2 are responsible for the prothrombinase and immunomodulatory activities of the molecule [Chan et al. Recombinant FGL-2 protein was previously shown to induce sprouting in vascular endothelial cells [Kim, I. When FGL-2 is expressed as a membrane-associated protein on activated macrophages and endothelial cells, it exhibits a coagulation activity capable of directly cleaving prothrombin to thrombin. FGL-2 accounts for the fibrin deposition and thrombosis associated with both experimental and human allograft rejection, which has been abrogated through the use of FGL-2 neutralizing antibodies or in FGL-2 knock out mice [Ghanekar, A. Hancock, W W et. Experimental data indicate that endothelial cells rather than leukocyte FGL-2 expression accounts for intravascular fibrin deposition [Ning et al. Interestingly, the authors observed that the normal tissue surrounding the tumor did not display overexpression of FGL-2, as observed in the tumor itself. Thus, the present invention relates to a method for the diagnosis or prognosis of a malignant proliferative disorder in a subject, through measuring FGL-2 prothrombinase activity, in a sample obtained from said subject. Essentially, detection of FGL-2 prothrombinase activity higher than control in a sample is indicative of the presence of a malignant disorder in said subject. Furthermore, when said activity remains high or increases over time in said subject, it is an indication of poor prognosis or resistance to therapy. In counterpart, decreasing FGL-2 prothrombinase activity over time indicates recovery for said subject. Thus, the present invention presents a prothrombinase enzyme for use in the diagnosis or prognosis of malignant proliferative disorders. In addition, the present invention provides a kit for diagnosis or prognosis of a malignant proliferative disorder, said kit comprising: Lane 1â€" bp DNA ladder marker from Promega. This finding suggested that FGL-2 prothrombinase activity may be used as a diagnostic as well as a prognostic marker for cancer. Thus, in a first, aspect, the present invention provides a method for the diagnosis of a malignant disorder in a subject, said method comprising the steps of: In one particular example, the present invention provides a method for the diagnosis of a malignant disorder in a subject, wherein said method comprises measuring FGL-2 prothrombinase activity in a blood sample from a subject, or in peripheral blood mononuclear cells PBMC , and comparing the activity measured in the sample from a subject with that of a control value; whereby a sample with FGL-2 prothrombinase activity higher than control is indicative of the presence of a malignant disorder in said subject. In a second aspect, the present invention also provides a method for the prognosis of a malignant proliferative disorder in a subject, said method comprising the steps of: In another aspect, the present invention provides a method for the diagnosis of malignancy in a sample, said method comprising the steps of: In one embodiment of the methods of the invention, said sample is a blood sample and comprises FGL-2 expressing cells, in which FGL-2 prothrombinase activity can be measured. In particular, said blood sample comprises PBMC. Any sample comprising mononuclear cells may be used in the methods described herein, including a cell culture-derived sample, e. As mentioned herein, prothrombinase activity is understood to be FGL-2 prothrombinase activity, since PBMC including monocytes and lymphocytes contain only FGL-2 and no other prothrombinase. Therefore addition of prothrombin specific substrate to the lysates of these cells containing FGL-2 specific enzyme is expected to generate thrombin specific end product. Measuring of FGL-2 prothrombinase activity is usually performed using the

thrombin generation assay, as described herein in the Examples, and as previously described, for example, by Gbanekar and colleagues [Ghanekar et al. Essentially, cells obtained from a sample are placed in contact with prothrombin obtained from a commercial source in assay conditions suitable for FGL-2 prothrombinase activity. Assay conditions are known to the man skilled in the art and are exemplified in the Methods herein below. Thrombin generation may be detected through the cleavage of its chromogenic substrate, which results in changing the color, meaning that enzymatic activity of the generated thrombin is detected and measured through its end product. Nevertheless, other methods for detecting thrombin could be envisioned, including immunological methods, which could measure the a mount of thrombin gene rated directly, using e. As exemplified herein below, thrombin generation may be measured at regular intervals, at least once, at least twice, or at least three times preferably twice at nm using an automated plate reader. Preferred time points are 0, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 60 min, 90 min, min, and min. Thrombin activity in each sample is calculated based on a comparison with the standard curve generated by known concentrations of thrombin. Thus, use of FGL-2 prothrombinase activity is herein contemplated for the detection and diagnosis of cancer per se, as well as in assessing prognosis of a cancer which has already been diagnosed. The present invention may also be useful in monitoring the efficacy of cancer treatment. Monitoring the efficacy of treatment is essential for assessing prognosis of cancer treatment. Hence, the diagnostic method presented herein may be effected in a subject either before, during or after cancer treatment, and the analysis of the results obtained at each time point the level of FGL-2 prothrombinase activity compared to that in the normal population. Cancer treatment, as referred to herein, relates to any treatment for eradicating a proliferative, disease, including radiotherapy, chemotherapy, etc. In a further aspect, the present invention provides a prothrombinase enzyme for use in the diagnosis detection or in evaluation of the prognosis of malignant disorders. Specifically, said diagnosis or prognosis of malignant disorders is effected through the measurement of said prothrombinase enzyme activity in a sample. In counterpart, a sample presenting a prothrombinase enzyme activity which is compatible, i. Prognosis of a malignant, disorder may be effected by measuring prothrombinase enzyme activity in a sample obtained on at least one time point throughout treatment. If a sample is obtained at only one time point, said time point, should be after treatment. An optimal procedure would involve measuring prothrombinase enzyme activity at least once before, during and after treatment of a malignant disorder. An elevation of said prothrombinase enzyme activity throughout time being indicative of no response to treatment or of poor prognosis, whereas a decrease of said prothrombinase enzyme activity throughout time being indicative of recovery or amelioration of the condition. Poor prognosis meaning that there is a worsening of the malignant disorder, which may, e. Examples of biological samples include body fluids and tissue specimens. The source of the sample may be blood, cerebrospinal fluid, tissue scrapings, swabs taken from body regions throat, vagina, ear, eye, skin, sores tissue, such as lymph nodes, or the like. Tissue specimens include tumor biopsies or biopsies from any organ, such as spleen, lymph nodes, liver, lung, mammary gland, pancreas, colon, uterus, skin, prostate, endocrine glands, esophagus, stomach, intestine, etc. In particular, a sample may be obtained from any lymphocyte-containing tissue. In another particular embodiment, said sample comprises peripheral blood mononuclear cells PBMC. As mentioned herein, said prothrombinase enzyme is FGL If the tissue is part of the lymphatic or immune systems, malignant cells may include, non-solid tumors of circulating cells. Malignancies of other tissues or organs may produce solid tumors. When referring herein to the term malignant proliferative disorder, or cancer, it includes solid and non-solid tumors. More particularly, solid tumors include carcinomas, sarcomas, melanomas and adenomas. Non-solid tumors that may be diagnosed by the method of the invention include, but are not limited to, myeloid leukemia such as chronic myelogenous leukemia, acute myelogenous leukemia, acute myelogenous leukemia with maturation, acute promyelocytic leukemia, acute non-lymphocytic leukemia, acute non-lymphocytic leukemia with increased basophiles, acute monocytic leukemia, acute myelomonocytic leukemia with eosinophilia, lymphocytic leukemia, such as acute lymphoblastic leukemia, chronic lymphocytic leukemia and myeloprobferative diseases. The methods described herein for detection, diagnosis,

assessment of prognosis, screening of cancer are suitable for any stage in cancer. In that regard, it is important to be familiar with the systems of staging cancer. Staging is based on knowledge of the way cancer develops. Cancer cells divide and grow without control or order to form a tumor. As the tumor grows, it can invade nearby organs and tissues. Cancer cells can also break away from the tumor and enter the bloodstream or lymphatic system. By moving through the bloodstream or lymphatic system, cancer can spread from the primary site to form tumors in other organs, which are denominated metastasis. For example, cancers of the brain and spinal cord are classified according to their cell type and grade. Different staging systems are also used for many cancers of the blood or bone marrow, such as lymphoma. However, other cancers of the blood or bone marrow, including most types of leukemia, do not have a clear-cut staging system. Another staging system, developed by the International Federation of Gynecology and Obstetrics, is used to stage cancers of the cervix, uterus, ovary, vagina, and vulva. This system uses the TNM format. The TNM staging system is as follows: T describes the si e of the tumor and whether it has invaded nearby tissue, N describes any lymph nodes that are involved, and M describes the presence of metastasis. Prostate cancer T2 N0 M0 means that the tumor is located only in the prostate and has not spread to the lymph nodes or any other part of the body. This system is used for all types of cancer. It groups cancer cases into five main categories: In situ is early cancer that is present only in the layer of cells in which it began; Localized is cancer that is limited to be organ in which it began, without evidence of spread; Regional is cancer that has spread beyond the original primary site to nearby lymph nodes or organs and tissues; Distant is cancer that has spread from the primary site to distant organs or distant lymph nodes: Unknown is used to describe cases for which there is not enough information to indicate a stage. Another commonly used staging system uses roman numerals: Carcinoma in situ early cancer that is present only in the layer of cells in which it began. The cancer has spread to another organ. In a further aspect the present invention provides a kit for diagnosis or prognosis of a malignant disorder, said kit comprising; a at least one reagent for measuring FGL-2 prothrombinase activity; and b instructions for measuring FGL-2 prothrombinase activity in a sample. The kit of the invention may further comprise: A non-limiting list, of reagents for measuring FGL-2 prothrombinase activity includes a prothrombinase substrate, prothrombin, buffers such as HEPES, Tris, or any other buffer suitable for prothrombinase reaction known in the art, any chromogenic substrate suitable for the determination of serine proteases, and any other necessary reagent known to the man skilled in the art. A non-limiting list of means for collecting a sample to be tested includes syringes, needles, in particular blood collection needles, sample tubes, and any other device necessary for collecting as sample known to the man skilled in the art. It is herein defined that said prothrombinase activity measured using the reagents provided b said kit represents FGL-2 prothrombinase activity. In one embodiment, any such kit is a kit comprising reagents for measuring FGL-2 prothrombinase activity. The kit may further optionally comprise any other necessary reagents such as detectable moieties, enzyme substrates and color reagents. The particular reagents and other components included in the diagnostic kit of the present invention can be selected from those available, in the art in accord with the specific diagnostic method practiced in the kit. The findings presented herein strongly suggest that the enhanced thrombin generation by mononuclear cells of cancer patients is the result of increased FGL-2 prothrombinase activity. One possible explanation to this phenomenon would be that malignant cells directly activate FGL-2 in monocytesilymphocytes. Thus, thrombin and pro-thrombinase activity, in particular FGL-2 prothrombinase activity, can be used as a marker of malignant process as well as a measure of disease extensiveness. The present invention is defined by the claims, the contents of which are to be read as included within the disclosure of the specification. Disclosed and described, it is to be understood that this invention is not limited to the particular examples, process steps, and materials disclosed herein as such process steps and materials may vary somewhat. The following Examples are representative of techniques employed by the inventors in carrying out aspects of the present invention. It should be appreciated that while these techniques are exemplary of preferred embodiments for the practice of the invention, those of skill in the art, in light of the present disclosure, will recognize that numerous modifications can be made without, departing from the

intended scope of the invention. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention is related. Textbooks describing such methods are, e.

Chapter 3 : Porcine fgl2 - Levy, Gary

April May June July August September October Cloning and characterization of hfgl2, the human counterpart to the mouse gene fgl2.

Field of the disclosure [] The disclosure relates to methods and uses for treating hepatic ischemia and reperfusion injury. In particular, the disclosure relates to interfering with the interaction of FGL2 with FcyRIIB to prevent or treat hepatic ischemia and reperfusion injury. A critical event during reperfusion injury is sinusoidal endothelial cell SEC death, which occurs within minutes of reperfusion and precedes hepatocyte death by several hours Natori ; Kohli ; Caldwell-Kenkel ; Ikeda Injury of the SEC after reperfusion causes microcirculatory disturbance associated with leukocyte and platelet adhesion and subsequent infiltration of inflammatory cells into the hepatic parenchyma Clavien ; Vajdova ; Panes ; Lemasters It has been demonstrated that apoptosis of SEC occurs within minutes after reperfusion and protection of SEC apoptosis prevents hepatocyte death and liver injury Kohli ; Natori ; Natori In contrast to the in vivo situation, the death of SEC in vitro occurs only after prolonged ischemia and 24hr of re-oxygenation. This suggests that reperfusion of the whole organ in vivo provides mediators, which rapidly activates the apoptotic cascade in SEC. FGL2 is expressed on the surface of different cell types, including endothelial cells Ghanekar , macrophages Liu , and T-cells Marazzi and is constitutively expressed in many organs including liver, lung, kidneys, and heart Ding ; Rychlik FcyReceptors are present on various cell populations including lymphocytes, macrophages, and SEC Xu ; Mousavi In another embodiment, the inhibitor comprises an antisense nucleic acid of a nucleic acid encoding FGL2. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the disclosure are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description. Brief description of the drawings [] The disclosure will now be described in relation to the drawings in which: Trichrome staining of wild type mice B-1 and FGL2 knockout mice B-2 following 60 minutes of ischemia and 24hr of reperfusion. Cleaved caspase 3 staining was present in the majority of SEC in wild type. Accordingly, in one embodiment, the animal is a mammal. In a particular embodiment, the animal is a human. The damage includes, without limitation, sinusoidal endothelial cell SEC death or apoptosis, which can be readily determined by a skilled person, for example, by detecting the presence of apoptotic markers, such as caspase-3 and propidium iodide. In one embodiment, the FGL2-FcyRIIB inhibitor is used or administered prior to or in conjunction with the liver surgery to prevent the onset of hepatic ischemia and reperfusion injury. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized i. In some suitable embodiments, the amount of a given compound or composition will vary depending upon various factors, such as the given drug or compound, the pharmaceutical formulation, the route of administration, the type of disease or disorder, the identity of the subject or host being treated, and the like, but can nevertheless be routinely determined by one skilled in the art. As defined herein, a therapeutically effective amount of a compound or composition of the present disclosure may be readily determined by one of ordinary skill by routine methods known in the art. The FGL2 protein may have any of the known published sequences for fgl2 which can be obtained from public sources such as GenBank. Examples of such sequences include, but are not limited to Accession Nos. The aforementioned sequences are incorporated herein by reference. The FGL2 protein can be obtained from any species, optionally a mammal including human and mouse. Examples of such sequences include but are not limited to Accession Nos. Substances having this property are identified readily using established in vitro and in vivo assays for measuring protein-protein interactions, for example, competitive binding assays, yeast two hybrid systems, coimmunoprecipitation, ELISA, Western Blot Analysis and Flow cytometry FACS. Such substances include, without limitation, antisense nucleic acid molecules, proteins, antibodies and fragments

thereof , small molecule inhibitors and other substances. In one embodiment, the inhibitor is targeted to the liver. A person skilled in the art would readily understand how to target to the liver. In another embodiment, haemoglobin haptoglobin coupling is used to target to the liver Levy et al. Antibodies can be fragmented using conventional techniques. Papain digestion can lead to the formation of Fab fragments. Suitable such antibodies include polyclonal antibodies, as well as monoclonal antibodies, and active fragments thereof. The antibodies can be non-human antibodies, but it is desirable to generate the more tolerated versions thereof such as chimeric and humanized antibodies. Antibodies so raised can then be screened using any of the protein-protein interaction assays noted above and established in the art, and antibodies that inhibit such interaction can be selected for use. The amino acid sequence for human FGL2, a useful immunogen for such purposes, is known from Levy et al. The preparation of rabbit polyclonal antiserum against FGL2 is described in Ding et al. Techniques for conferring immunogenicity on a peptide include conjugation to carriers or other techniques well known in the art. For example, the protein or peptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay procedures can be used with the immunogen as antigen to assess the levels of antibodies. Following immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera. Such techniques are well known in the art, e. Today 4, 72 , the EBV-hybridoma technique to produce human monoclonal antibodies Cole et al. Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the peptide and the monoclonal antibodies can be isolated. Chimeric antibody molecules can include, for example, the antigen binding domain from an antibody of a mouse, rat, or other species, with human constant regions. It is expected that chimeric antibodies would be less immunogenic in a human subject than the corresponding non-chimeric antibody. Such immunoglobulin molecules may be made by techniques known in the art, e. For example, complete Fab fragments, VH regions and FV regions can be expressed in bacteria using phage expression libraries See for example Ward et al. Nature , Alternatively, a SCID-hu mouse, for example the model developed by Genpharm, can be used to produce antibodies or fragments thereof. Peptide mimetics also include molecules incorporating peptides into larger molecules with other functional elements e. Peptide mimetics also include peptoids, oligopeptoids Simon et al Proc. Acad, Sci USA Local conformational constraints can also be introduced to determine conformational requirements for activity of a candidate peptide mimetic. The mimetics may include isosteric amide bonds, or D-amino acids to stabilize or promote reverse turn conformations and to help stabilize the molecule. Cyclic amino acid analogues may be used to constrain amino acid residues to particular conformational states. The mimetics can also include mimics of the secondary structures of the proteins described herein. These structures can model the 3-dimensional orientation of amino acid residues into the known secondary conformations of proteins. Peptoids may also be used which are oligomers of N-substituted amino acids and can be used as motifs for the generation of chemically diverse libraries of novel molecules. The term "oligonucleotide" refers to an oligomer or polymer of nucleotide or nucleoside monomers consisting of naturally occurring bases, sugars, and intersugar backbone linkages. The term also includes modified or substituted oligomers comprising non-naturally occurring monomers or portions thereof, which function similarly. Such modified or substituted oligonucleotides may be preferred over naturally occurring forms because of properties such as enhanced cellular uptake, or increased stability in the presence of nucleases. The term also includes chimeric oligonucleotides which contain two or more chemically distinct regions. For example, chimeric oligonucleotides may contain at least one region of modified nucleotides that confer beneficial properties e. The oligonucleotides may also contain modified bases such as xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza uracil, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiouracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8- halo guanines, 8-amino guanine, 8-thiol guanine, 8-thiolalkyl guanines, 8- hydroxyl guanine and other 8-substituted guanines, other aza and deaza uracils, thymidines, cytosines, adenines, or guanines, 5-trifluoromethyl uracil and

5-trifluoro cytosine. For example, the antisense oligonucleotides may contain phosphorothioates, phosphotriesters, methyl phosphonates, and phosphorodithioates. In another embodiment phosphorothioate bonds link all the nucleotides. An example of an oligonucleotide analogue is a peptide nucleic acid PNA wherein the deoxyribose or ribose phosphate backbone in the DNA or RNA , is replaced with a polyamide backbone which is similar to that found in peptides P. Science , , PNA analogues have been shown to be resistant to degradation by enzymes and to have extended lives in vivo and in vitro. Other oligonucleotides may contain nucleotides containing polymer backbones, cyclic backbones, or acyclic backbones. For example, the nucleotides may have morpholino backbone structures U. Oligonucleotides may also contain groups such as reporter groups, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an antisense oligonucleotide. Antisense oligonucleotides may also have sugar mimetics. The antisense nucleic acid molecules of the disclosure or a fragment thereof, may be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed with mRNA or the native gene e. The antisense sequences may be produced biologically using an expression vector introduced into cells in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense sequences are produced under the control of a high efficiency regulatory region, the activity of which may be determined by the cell type into which the vector is introduced. The antisense oligonucleotides may be directly administered in vivo or may be used to transfect cells in vitro which are then administered in vivo. Methods of designing specific siRNA molecules and administering them are known to a person skilled in the art. Adding two thymidine nucleotides is thought to add nuclease resistance. A person skilled in the art will recognize that other nucleotides can also be added. Aptamers are short strands of nucleic acids that can adopt highly specific 3- dimensional conformations. Aptamers can exhibit high binding affinity and specificity to a target molecule. These properties allow such molecules to specifically inhibit the functional activity of proteins. Various constructs can be used to deliver nucleic acid molecules described herein. For example retroviral constructs such as lentiviral constructs are useful for expressing physiological levels of protein. Possible expression vectors include but are not limited to cosmids, plasmids, or modified viruses e. The expression vectors are "suitable for transformation of a host cell", which means that the expression vectors contain a nucleic acid molecule and regulatory sequences selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid molecule. Operatively linked is intended to mean that the nucleic acid is linked to regulatory sequences in a manner which allows expression of the nucleic acid. Selection of appropriate regulatory sequences is dependent on the host cell chosen as discussed below, and may be readily accomplished by one of ordinary skill in the art. Examples of such regulatory sequences include: Additionally, depending on the host cell chosen and the vector employed, other sequences, such as an origin of replication, additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector. If the selectable marker gene encodes a protein conferring antibiotic resistance such as neomycin resistance transformant cells can be selected with G Cells that have incorporated the selectable marker gene will survive, while the other cells die. This makes it possible to visualize and assay for expression of the recombinant expression vectors disclosed herein and in particular to determine the effect of a mutation on expression and phenotype. It will be appreciated that selectable markers can be introduced on a separate vector from the nucleic acid of interest. For example, the proteins of the disclosure may be expressed in bacterial cells such as E. As used herein, "contemporaneous administration" of two substances to an individual means providing each of the two substances so that they are both biologically active in the individual at the same time.

Chapter 4 : USA1 - Compositions comprising antibodies to human fgl2 - Google Patents

The rat Fgl-2 amino acid sequence was compared with the known sequences in mouse and calendrierdelascience.coms:Fgl-w specific amplicon bands were observed in the rat brain, kidney, liver, ovary, spleen, and gestational day 22 and postpartum uterus.

They can be in liquid, solid or semisolid form, for example pills, tablets, creams, gelatin capsules, capsules, suppositories, soft gelatin capsules, gels, membranes, tubelets, solutions or suspensions. Dosages to be administered depend on individual needs, on the desired effect and on the chosen route of administration. The pharmaceutical compositions may additionally contain other agents such as adjuvants to enhance immune responsiveness. Recombinant molecules comprising an antisense sequence or oligonucleotide fragment thereof, may be directly introduced into cells or tissues in vivo using delivery vehicles such as retroviral vectors, adenoviral vectors and DNA virus vectors. They may also be introduced into cells in vivo using physical techniques such as microinjection and electroporation or chemical methods such as coprecipitation and incorporation of DNA into liposomes. Recombinant molecules may also be delivered in the form of an aerosol or by lavage. The antisense nucleic acid molecules of the invention may also be applied extracellularly such as by direct injection into cells. In one embodiment, the vaccine may comprise a peptide having amino acids to shown in FIG. The vaccine may be useful in preventing graft rejection when administered prior to or concurrently with a transplant. Examples of adjuvants include the lipid A portion of gram negative bacteria undotoxin, trehalose dimycolate of mycobacteria, the phospholipid lysoleathin, dimethyl dictadecyl ammonium bromide DDA , linear polyoxypropylene-polyoxyethylene POP-POE block polymers and liposomes. The vaccine may also contain preservatives such as sodium azide, thimersol, beta propiolactone, and binary ethyleneimine. The dosage will depend on the nature of the disease, on the desired effect and on the chosen route of administration, and other factors known to persons skilled in the art. A portion of an Fgl2 gene preferably includes a nucleic acid molecule encoding a peptide comprising the amino acid residues at positions to in FIG. In-vitro Testing and Animal Models [] The utility of the inhibitors, antibodies, antisense nucleic acid molecules, Fgl2 protein and nucleic acid molecules and compositions of the invention may be confirmed in in vitro systems and animal model systems. For example, the following concordant and discordant models may be used: Tissues may be examined for ability of monoclonal antibodies to prevent fibrin disposition, platelet adherence and cellular infiltration. Control animals will receive an irrelevant antibody of similar isotope. Pig to primate experiments may be conducted using similar protocols. Cells, tissues, and non-human animals lacking in expression or partially lacking in expression of the protein may be developed using recombinant molecules of the invention having specific deletion or insertion mutations in the nucleic acid molecule of the invention. A recombinant molecule may be used to inactivate or alter the endogenous gene by homologous recombination, and thereby create a deficient cell, tissue or animal. Such a mutant cell, tissue or animal may be used to define specific cell populations, developmental patterns and in vivo processes, normally dependent on the protein encoded by the nucleic acid molecule of the invention. By way of example, a targeted recombination strategy may be used to inactivate the endogenous fgl2 gene. A gene which introduces stop codons in all reading frames and abolishes the biological activity of the prothrombinase may be inserted into a genomic copy of the fibrinogen like protein. The effects of the mutation on immune response allo and xeno transplantation, viral hepatitis in comparison to non-mutated controls may be determined, and the survival and histologic pattern of disease may be analyzed. The probes can be useful in detecting the presence of a condition associated with immune coagulation or monitoring the progress of such a condition. Accordingly, the present invention provides a method for detecting a nucleic acid molecules encoding Fgl2 comprising contacting the sample with a nucleotide probe capable of hybridizing with the nucleic acid molecule to form a hybridization product, under conditions which permit the formation of the hybridization product, and assaying for the hybridization product. A nucleotide probe may be labelled with a

detectable substance such as a radioactive label which provides for an adequate signal and has sufficient half-life such as 32P, 3H, 14C or the like. Other detectable substances which may be used include antigens that are recognized by a specific labelled antibody, fluorescent compounds, enzymes, antibodies specific for a labelled antigen, and chemiluminescence. An appropriate label may be selected having regard to the rate of hybridization and binding of the probe to the nucleic acid to be detected and the amount of nucleic acid available for hybridization. Labelled probes may be hybridized to nucleic acids on solid supports such as nitrocellulose filters or nylon membranes as generally described in Sambrook et al, , Molecular Cloning, A Laboratory Manual 2nd ed. The nucleotide probes may be used to detect genes, preferably in human cells, that hybridize to the nucleic acid molecule of the present invention preferably, nucleic acid molecules which hybridize to the nucleic acid molecule of the invention under stringent hybridization conditions as described herein. It is possible to design synthetic oligonucleotide primers from the nucleotide sequence shown in FIGS. The amplified nucleic acid can be cloned into an appropriate vector and characterized by DNA sequence analysis. Accordingly, the present invention provides a method for detecting a Fgl2 protein comprising contacting the sample with an antibody that binds to Fgl2 which is capable of being detected after it becomes bound to the Fgl2 in the sample. Thus, the antibodies may be used to quantify the amount of the protein in a sample in order to determine its role in particular cellular events or pathological states and to diagnose and treat such pathological states. Generally, an antibody specific for the protein may be labelled with a detectable substance as described herein and the protein may be localised in tissue based upon the presence of the detectable substance. FGL2 Genes and Proteins [] As hereinbefore mentioned, the present inventor has cloned and sequenced genomic hFgl2. In this regard, the entire genomic sequence as well as the sequence of the promoter region, shown in FIG. The fragments generally comprise a nudeic acid sequence having at least 15 bases which will hybridize to the sequences shown in FIGS. Such conditions are known to those skilled in the art and are described, for example, in Sambrook, et al, , Molecular Cloning, A Laboratory Manual, Cold Spring Harbor. By way of example only, stringent hybridization with short nucleotides may be carried out at below the T m using high concentrations of probe such as 0. The present invention in particular contemplates nucleic acids encoding the amino acids at positions to , preferably to in the amino acid sequence shown in FIG. It will also be appreciated that variant forms of the nucleic acid molecules of the invention which arise by alternative splicing of an mRNA corresponding to a cDNA of the invention are encompassed by the invention. It is also contemplated that nucleic acid molecules of the invention will be prepared having mutations such as insertion or deletion mutations, e. The variations may be attributable to local mutations or structural modifications. Such nucleic acids encode functionally equivalent proteins e. However, DNA sequence polymorphisms may lead to changes in the amino acid sequences of human Fgl2 within a population. Such nucleotide variations and resulting amino acid polymorphisms are within the scope of the invention. For example, a labelled nucleic acid probe based on all or part of the nucleic acid sequence shown in FIG. For example, a cDNA can be cloned downstream of a bacteriophage promoter, e. Various methods of chemically synthesizing polydeoxynucleotides are known, including solid-phase synthesis which, like peptide synthesis, has been fully automated in commercially available DNA synthesizers See e. A cDNA having the biological activity of human Fgl2 so isolated can be sequenced by standard techniques, such as dideoxynucleotide chain termination or Maxam-Gilbert chemical sequencing, to determine the nucleic acid sequence and the predicted amino acid sequence of the encoded protein. The intron-exon structure and the transcription regulatory sequences of the gene encoding human Fgl2 may be identified by using a nudeic acid molecule of the invention encoding human Fgl2 to probe a genomic DNA clone library. Regulatory elements can be identified using conventional techniques. The function of the elements can be confirmed by using them to express a reporter gene such as the bacterial gene lacZ which is operatively linked to the elements. These constructs may be introduced into cultured cells using standard procedures or into non-human transgenic animal models. Such constructs may also be used to identify nuclear proteins interacting with the elements, using techniques known in the art. A truncated Fgl2 protein or fragment of the human Fgl2 protein is a portion of the full-length Fgl2

amino acid sequence having one or more amino acid residues deleted. The deleted amino acid residue s may occur anywhere in the polypeptide, including at either the N-terminal or C-terminal end or internally. Fgl2 fragments typically will have a consecutive sequence of at least 10, 15, 20, 25, 30, or 40 amino acid residues that are identical to the sequences of the human Fgl2. The truncations or portions of the Fgl2 protein may comprise an antigenic site that is capable of cross-reacting with antibodies raised against the Fgl2 protein whose sequence is shown in FIG. Therefore, immunogenic portions or fragments of human Fgl2 proteins are within the scope of the invention e. The truncated proteins may have a carboxyl group, an amido group, a T-butyloxycarbonyl group, or a macromolecule including lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates at the carboxy terminal end. Amino acid substitutions may be of a conserved or non-conserved nature. When only conserved substitutions are made the resulting analog should be functionally equivalent to human Fgl2 as described herein. Amino acid insertions may consist of single amino acid residues or sequential amino acids ranging from 2 to 15 amino acids in length. For example, amino acid insertions may be used to destroy the prothrombinase activity of the protein. The deleted amino acids may or may not be contiguous. The lower limit length of the resulting analog with a deletion mutation is about 10 amino acids, preferably amino acids. Such homologs are proteins whose amino acid sequences are comprised of the amino acid sequences of human Fgl2 regions from other species that hybridize under stringent hybridization conditions see discussion of stringent hybridization conditions herein with a probe used to obtain human Fgl2 as shown in FIG. An isoform contains the same number and kinds of amino acids as the protein of the invention, but the isoform has a different molecular structure. The isoforms contemplated by the present invention are those having the same properties as the protein of the invention as described herein. Accordingly, the nucleic acid molecules of the present invention or a fragment thereof may be incorporated in a known manner into an appropriate expression vector which ensures good expression of the protein. Possible expression vectors include but are not limited to cosmids, plasmids, or modified viruses, so long as the vector is compatible with the host cell used. Suitable transcription and translation elements may be derived from a variety of sources, including bacterial, fungal, viral, mammalian, or insect genes. Selection of appropriate transcription and translation elements is dependent on the host cell chosen as discussed below, and may be readily accomplished by one of ordinary skill in the art. Examples of such elements include: Additionally, depending on the host cell chosen and the vector employed, other genetic elements, such as an origin of replication, additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector. This makes it possible to visualize and assay for expression of recombinant molecules of the invention and in particular to determine the effect of a mutation on expression and phenotype. Methods for transforming transfecting, etc. This may be accomplished, for example, by the synthesis of N-terminal or C-terminal fusion proteins. Thus, fusion proteins may be prepared by fusing, through recombinant techniques, the N-terminal or C-terminal of the protein, and a selected protein with a desired biological function. The resultant fusion proteins contain the protein or a portion thereof fused to the selected protein. Cloning The Human Prothrombinase Gene hfgl2: Three clones, namely , , and were found positive for this screening. The plasmids containing these three clones were purified using the Qiagen maxiprep DNA purification protocol. The quality of the purified DNA and the presence of the inserts were verified by digesting the plasmid with Not 1 restriction enzyme Canadian Life, Burlington, Canada , and subjecting the samples to Clamped Homogenous Electric Field CHEF gel electrophoresis , at angle, 6 Volts, seconds ramp interval, 0. In order to reduce the DNA into fragments of 5 to 10 kb, which is a convenient size range to work with, the clone was digested under sub-optimal conditions with the restriction enzyme Sau 3A Canadian Life, Burlington, Canada. The final products of the restriction digest were subjected to CHEF gel electrophoresis at the above conditions. This Bluescript II library was screened using the huflp1 and 2 primers. The primers were labeled at the 5 end with gamma P32 by using the enzyme Polynucleotide Kinase Pharmacia, Uppsala, Sweden ; these primers were used to screen the Bluescript library. The clone J14 hybridized to both these primers and was used for the subsequent work. The sequence was read from the

autoradiograph using the Helixx sequence reading equipment Helixx Technologies Inc. New primers are designed based on the outcomes of manual sequencing and the published cDNA sequence. The coding region has been extensively analyzed and compared to the mouse gene. In order to gather insight into the functional properties of this gene, the protein sequence was predicted from the genomic sequence and compared to mouse fgl2 direct prothrombinase protein and other relevant coagulation proteases. The first nucleotide of the reported cDNA is considered the putative transcription start site. In the hfgl2 cDNA, the first nucleotide is a cytosine, and the transcription initiation site does not comply to the above consensus. In the coding region there are long stretches of conserved sequences.

Chapter 5 : Methods of modulating immune coagulation - LEVY GARY

The Fgl2/fibroleukin genotype failed to modify this response. Also, there was no difference in survival between non-C3H/HeJ strains as seen by Kaplan Meier analysis, demonstrating that disruption of the fgl2 gene does not affect systemic LPS responses (Figure (Figure3 3 b).

See other articles in PMC that cite the published article. Typically, mFGL2 functions as prothrombinase that is capable of initiating coagulation in tissue without activation of the blood clotting cascade, whereas sFGL2 largely acts as an immunosuppressor that can repress proliferation of alloreactive T lymphocytes and maturation of bone marrow dendritic cells. Protein sequences of FGL2 exhibit evolutionary conservation across wide variety of species, especially at the carboxyl terminus that contains fibrinogen related domain FRED. Constitutive expression of FGL2 during embryogenesis and in mature tissues suggests FGL2 might be physiologically important. However, excessive induction of FGL2 under certain medical conditions e. On the other hand, complete absence of FGL2 is also detrimental as lack of FGL2 can cause autoimmune glomerulonephritis and acute cellular rejection of xenografts. Although it is not clear how mFGL2 is cleaved off its host cells and secreted into the blood, circulating sFGL2 has been found correlated with disease severity and viral loading among patients with human hepatitis B virus or hepatitis C virus infection. Further studies are warranted to understand how FGL2 expression is regulated under physiological and pathological conditions. Even more interesting is to determine whether mFGL2 can fulfill an immunoregulatory role through its FRED at carboxyl end of the molecule and, and vice versa, whether sFGL2 is procoagulant upon binding to a target cell. Knowledge in this area should shed light on development of sFGL2 as an alternative immunosuppressive agent for organ transplantation or as a biomarker for predicting disease progression, monitoring therapeutic effects, and targeting FGL2 for repression in ameliorating fulminant viral hepatitis. FGL2 has been found to be not only physiologically important[1 â€” 3], but also involved in pathogenesis of viral infections[4 , 5], pregnancy failure[6], autoimmune disorders[7 , 8], allograft rejections[9], and tumor growth[10]. Alternations in FGL2 expression or structure are tied to several highly virulent viral infections, including human immunodeficiency virus HIV infection, severe acute respiratory syndrome SARS , and hepatitis B and C[4 , 5 , 11]. In this review, constitutive expression and physiological roles of FGL2 that have been identified to date will be illustrated to help understand pathological properties of FGL2 during pathogen invasion when ectopic expression of FGL2 occurs. While FGL2 might have a potential to be used as a biomarker or therapeutic target, some research gaps will be explored to expand possible clinical applications of FGL2. Although signal transduction pathways involved in regulation of FGL2 transcription and post-transcriptional modifications are important, in-depth discussion of these molecular mechanisms is not in the scope of this review. A phylogenetic tree analysis suggests an even closer evolutional relationship between human Fgl2 and pig Fgl2[16]. This extraordinary evolutionary conservation across different species suggests that FGL2 might be an indispensable protein with critical biological function s. Three tentative serine protease active sites at positions 91, , and are conserved between human and mouse FGL2[14]. The residue serine 91 of human FGL2, corresponding to the serine 89 of murine FGL2, has been revealed to be capable of cleaving prothrombin into thrombin [14 , 22]. FGL2 was thus speculated and has been demonstrated to function as a prothrombinase[15 , 22 , 23]. FGL2 activates prothrombin to generate thrombin that in turn converts fibrinogen into fibrin, a process equivalent to factor II F II activation[15 , 22 , 23]. Instead, full proteolytic activity of FGL2 is contingent on its physical association with membrane phospholipids, factor Va, and calcium[22]. Therefore, prothrombinase activity appears to be intrinsic to mFGL2, but similar function of sFGL2, if there is any, has yet to be demonstrated. A stretch of hydrophobic amino acids at N-terminus of FGL2 may serve as signal peptide for sFGL2 secretion[14], but the mechanism whereby sFGL2 is cleaved and released outside of the host cell remains to be determined. Glycosylation of the amino acids at positions of , , , and were found to be critical to maintain the solubility of sFGL2[16]. Previous studies indicate that

sFGL2 lacks procoagulant activity of mFGL2[12 , 17] , rather, it functions largely as an immunosuppressor or pro-apoptotic effector molecule[26]. This receptor is expressed on sinusoidal endothelial cells SECs within the liver[28]; glomerular mesangial cells within the kidney[29]; and immunoregulatory cells such as dendritic cells DCs , B lymphocytes, macrophages, and activated T lymphocytes[16 , 30 , 31]. Binding of sFGL2 to SECs, glomerular mesangial cells, B lymphocytes, or macrophages caused apoptosis of the target cells[8 , 28 , 30]. Consistent with these observations, the levels of Th2 cytokines and the activity of DCs, B lymphocytes, and T lymphocytes have all been found to be increased in FGL2-deficient mice[8]. Constitutive expression of FGL2 has been detected in the heart, lung, small bowel, spleen, ovary, uterus, liver, and kidney[13 , 32]. Expression of FGL2 is regulated tightly and associated with several physiological processes, including sperm maturation[1], embryo development[3 , 33], and smooth muscle contraction[2 , 34]. FGL2 might play a protective role during sperm maturation in epididymis[1]. The expression of Fgl2 messenger RNA mRNA under normal physiological conditions has been identified in the tubule principal cells of hamster epididymis[1]. FGL2 was found to be secreted from the principal cells into the tubule lumen where sFGL2 binds specifically to the nonviable, but not the viable, spermatozoa[1]. This process forms sFGL2-protein complex that coats and envelops dying sperms to restrict release and spread of detrimental enzymes and immunogenic molecules from defective spermatozoa. Nevertheless, FGL2-deficient male mice were still fertile[35], suggesting that the potential protective role of FGL2 is limited, or becomes prominent only under certain medical conditions when increased apoptosis of spermatozoa occurs. Expression of FGL2 has been shown to change dynamically during murine embryogenesis[3]. FGL2 was first detectable at the implantation site at E5. The physiological function of FGL2 has been further demonstrated in animal studies. Early miscarriage of mouse embryos between the time of implantation E4. In addition, mFGL2 might function as prothombinase to improve coagulation and reduce hemorrhage at the implantation site that is often seen in FGL2-deficient embryos but not in wild type embryos[3 , 23 , 38]. FGL2 might be involved in modulation of vascular and nonvascular smooth muscle contraction. Expression of FGL2 was detected in mouse cardiomycocytes[2]. These data suggest that FGL2 is critical for normal myocardial function during prenatal and postnatal development in mice[2], but it is not clear how FGL2 deficiency is linked to abnormal myocardial contraction. Increased level of FGL2 can lead to thrombin accumulation in myometrium. Thrombin in turn binds to these receptors and causes cytosolic enrichment of calcium[39]. This process may ultimately result in myometrial smooth muscle contraction[39 , 40]. Pretreatment with a thrombin-specific inhibitor hirudin prevented myometrial contraction[40]. Therefore, FGL2 appears to modulate vascular and nonvascular muscle contraction through generation of thrombin. This section focuses on potential mechanisms behind the pathogenesis of the infectious diseases that involve abnormal activities of mFGL2, sFGL2, or both. HIV-1 infection typically advances through acute phase to asymptomatic stages and finally to full-blown acquired immune deficiency syndrome AIDS. Acute stage is characterized by elevated expression of genes involved in immune activation and defenses, resulting in partial control of HIV infection and progression to asymptomatic stage. Expression of a host of immunosuppressive genes including FGL2 is activated at the asymptomatic stage[5]. It is worth noting that the G53E point mutation is not located near known important functional motifs, including FRED, glycosylation sites, and serine prothrom-binase sites. There is no report to date exploring whether and how the G53E mutation might affect the function of sFGL2. However, Siu et al[48] was not able to reproduce the results in the Vero cell line, nor in human embryonic kidney cells or cultured human airway epithelial cells. Strong fibrin deposition and necrosis were co-localized with robust FGL2 expression in liver biopsies from 21 out of 23 patients with acute-on-chronic hepatitis B characterized by recurrent flares of hepatocellular injury, but not among all 13 patients with minimal chronic hepatitis that exhibited no major active liver pathology[23 , 51]. The expression of FGL2 seemed to correlate with the progression of viral hepatitis. Fibrosis stage is an established indication for disease severity and efficacy of anti-viral treatment. However, the levels of FGL2 among HCV-patients with inactive alcoholic cirrhosis were comparable to the controls[4], suggesting that it is the activity and progression of HCV infection, not the end

stage cirrhosis, that accounts for the high levels of FGL2 among HCV-patients. Han et al[11] found that HBV core protein or X protein were both capable of binding directly to the promoter of FGL2 gene and activating its transcription in a hepatocellular carcinoma cell line. High levels of sFGL2 in turn might jeopardize the host immune integrity and thus may facilitate viral replication and expansion. In addition, FGL2-mediated accumulation of fibrin could restrain or block blood flow in the liver and cause hepatocyte necrosis or even liver failure[23 , 51]. Observations from animal studies have provided further insights into the pathogenesis of viral hepatitis. Mouse hepatitis virus 3 MHV3 -a member of Corona-viridae-has served as a model for dissecting pathological determinants of diseases caused by coronaviruses. FGL2 protein was detected within 24 h following MHV3 infection in endothelium of intrahepatic veins and hepatic sinusoids where concomitant fibrin deposition and subsequent focal hepatocyte necrosis occurred[56]. Ning et al[57 , 58] demonstrated that the nucleocapsid protein of MHV3 was capable of activating transcription of mouse Fgl2 gene in vitro. Further, they have showed that administration of anti-FGL2 monoclonal antibody mAb improved liver histology and survival rate[27]. However, several lines of evidence suggest that FGL2-mediated immunosuppression is not a major determinant in the pathogenesis of viral hepatitis. Second, although administration of FGL2 neutralizing antibodies abrogated hepatitis in mice infected by MHV3, a high viral load persisted[60]. Recent studies have demonstrated that the progression of fulminant viral hepatitis usually exhibits a similar pattern, viral-induced up-regulation of the Fgl2 gene precedes focal deposits of fibrin in sinusoids, followed by accumulation of inflammatory cells and focal hepatocyte necrosis. The roles of FGL2 in the rapid development of confluent multicellular hepatic necrosis are probably fulfilled through several interrelated processes: Thrombin is known to be able to stimulate endothelial cells to produce IL-8[62 , 63]. IL-8 is a potent chemo-attractant for polymorphonuclear leukocytes that have been identified at the sites of FGL2-mediated inflammation. MHV3 is propagated in macrophages[19]. Additionally, macrophages are a source of FGL2 production[19 , 56 , 64]. Therefore, reduction in the number of macrophages due to apoptosis might translate into a diminished level of FGL2. This finding appears to be clinically relevant. Taken together, the level of FGL2 correlates positively with the development and severity of typical MHV cytopathology[51 , 64]. Animal studies have suggested that elevation of FGL2 might be one of critical determinants of susceptibility to hepatitis virus infection[51]. FGL2 appears to be such a candidate. Variations in the plasma level of FGL2 among healthy human volunteers were minimal, regardless of race, gender, or age[4]. In contrast, plasma levels of FGL2 correlated positively with HCV titers and degree of inflammation in the liver[4]. Furthermore, as discussed previously, FGL2 expression has been found to be associated with progression and severity of disease. FGL2-procoagulant activity was more than fold higher on PBMCs from patients with acute-on-chronic hepatitis B than from healthy controls[51]. Plasma levels of FGL2 have been found to correlate with diseases other than viral hepatitis. For example, the levels of plasma FGL2 were significantly higher among patients with fatty liver disease than healthy controls[69]. Likewise, although the elevation of FGL2 was not associated with clinical features of systemic sclerosis, the mean serum level of FGL2 among patients with systemic sclerosis Recent research has provided exciting insight into clinical application of FGL2 as a therapeutic target. Animal studies suggested that effective disease intervention could be achieved through modulation of FGL2 expression at DNA or protein level. All 18 mice receiving Fgl2 antisense plasmid were alive on 3 d post MHV3-infection[71]. Similar effects have been observed by targeting FGL2 protein directly. Administration of FGL2-mAb resulted in a dose-dependent reduction of MHV3 viral titers among infected mice and improved liver histology and survival rate[27 , 60]. Infections after organ transplantation remain a significant cause of mortality among the recipients[72 , 73]. Current steroid or steroid-free immunosuppression scheme following an organ transplantation has been found to be associated with cardiovascular disease and infections[72 , 73 , 75]. Therefore, novel regimen is in great need to overcome or minimize adverse effects of immunosuppression[30]. Intravenous injection of recombinant sFGL2 into donor mice receiving skin transplantation prolonged the survival of skin allografts from 7. This finding might be clinically significant in that FGL2 could induce immune tolerance without

relying on prolonged immunosuppression and thus help to reduce the risk of development of cardiovascular disease, infections, or cancer. Interestingly, monomeric FGL2 has been found to exhibit greater immunosuppressive activity than native oligomer sFGL2[16].