PROTEIN S ANTIBODIES, METHODS OF MAKING AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/020,505, filed on May 5, 2020 and to U.S. Provisional Patent Application No. 63/169,755 filed April 1, 2021. The contents of each of the preceding applications are incorporated by reference herein in their entirety.

BACKGROUND

[0002] Protein S (also known as ProS, ProS1) is a vitamin K-dependent plasma protein involved in the anti-coagulation cascade. The protein is multi-modular, comprising a γ-carboxy-glutamic acid domain (Gla domain), an epidermal growth factor-like domain (EGF domain), a thrombin-sensitive region (TSR), and a sex hormone binding globulin-like domain (SHBG-like domain). The protein is found in both a free form and as part of a complex with proteins such as C4 binding protein (C4BP) and tissue factor pathway inhibitor (TFPI). Among other functions, in its free form, Protein S is a cofactor in at least two pathways of the anti-coagulation cascade: (1) Protein S is a cofactor for plasma activated Protein C (APC), involved in the inactivation and degradation of coagulation factors Factor Va and Factor VIIIa; and (2) Protein S is also a cofactor for TFPI, also present in plasma, involved in the inactivation of coagulation factors Factor Xa and Factor VIIa.

[0003] Protein S is a potential therapeutic target for bleeding disorders, thus there is a need for agents that bind and modulate its activities within the coagulation pathway.

SUMMARY

[0004] Provided here are antibodies that bind Protein S, and methods of making and using such antibodies. In some examples, the Protein S antibodies provided herein are useful for treating a bleeding disorder, or a condition characterized by reduced or impaired blood coagulation and/or clotting.

[0005] Accordingly, in one example, provided herein are antibodies that bind Protein S, wherein the antibodies are inhibitors of the cofactor activity of Protein S for activated Protein C (APC), inhibitors of the cofactor activity of Protein S for tissue factor pathway inhibitor (TFPI), or

inhibitors of the cofactor activity of Protein S for both APC and TFPI (dual inhibitor of cofactor activity), and wherein the antibody is human, humanized, or chimeric. In some preferred examples, the antibodies provided herein specifically bind Protein S.

[0005a] In one aspect, the present disclosure refers to an antibody that binds Protein S, wherein the antibody is an inhibitor of the cofactor activity of Protein S for activated Protein C (APC), an inhibitor of the cofactor activity of Protein S for tissue factor pathway inhibitor (TFPI), an inhibitor of the cofactor activity of Protein S for both APC and TFPI, or is capable of promoting coagulation and/or modulating a component in the coagulation cascade and wherein the antibody is human, humanized, or chimeric.

[0006] In another example, provided herein are antibodies that bind Protein S, wherein the antibodies are capable of promoting coagulation and/or modulating a component in the coagulation cascade.

[0007] In another example, provided herein are exemplary Protein S antibodies comprising any one or more of the amino acid sequences of the complementarity determining region (CDR) sequences provided in Tables 1A, 1B, 1C, 2A, 2B, and 2C. In another example, the exemplary Protein S antibodies comprise any one of the CDR-L1 amino acid sequences of Table 1A; any one of the CDR-L2 amino acid sequences of Table 1B; any one of the CDR-L3 amino acid sequences of Table 1C; any one of the CDR-H1 amino acid sequences of Table 2A; any one of the CDR-H2 amino acid sequences of Table 2B; any one of the CDR-H3 amino acid sequences of Table 2B. In another example, provided herein are exemplary Protein S antibodies comprising the combinations of variable light chains and variable heavy chains presented in Table 4C. In another example, provided herein are the sequences of different antibodies as presented in Table 6. In another example, provided herein are nucleic acids encoding for any of the Protein S antibodies provided herein.

[0008] In another example, provided herein are pharmaceutical compositions comprising any one of the Protein S antibodies provided herein, and optionally a pharmaceutically acceptable excipient.

[0008a] In one aspect, the present disclosure refers to a pharmaceutical composition comprising the antibody disclosed herein, and optionally a pharmaceutically acceptable excipient. In one aspect, the present disclosure refers to a nucleic acid encoding for the antibody disclosed herein. In one aspect, the present disclosure refers to a vector comprising the nucleic acid disclosed herein.

[0009] Provided herein are methods of using the exemplary Protein S antibodies described herein. Accordingly, in one example, provided herein is an *in vitro* method of promoting the coagulation of blood, comprising contacting any one of the Protein S antibodies provided herein with a blood sample.

[0009a] In one aspect, the present disclosure refers to an *in vitro* method of promoting the coagulation of blood, comprising contacting the antibody disclosed herein with a blood sample.

[0010] In another example, provided herein is a method of promoting the coagulation of blood in a subject in need thereof, comprising administering to the subject any of the exemplary Protein S antibodies provided herein, or pharmaceutical compositions provided herein. In examples, the route of administration is subcutaneous.

[0010a] In one aspect, the present disclosure refers to use of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for promoting the coagulation of blood in a subject in need thereof.

[0011] In another example, provided herein is a method of promoting the generation of thrombin in a subject in need thereof, comprising administering to the subject any of the Protein S antibodies provided herein, or pharmaceutical compositions provided herein.

[0011a] In one aspect, the present disclosure refers to use of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for promoting the generation of thrombin in a subject in need thereof.

[0012] In another example, provided herein is a method of treating a condition in a subject in need thereof, comprising administering to the subject any of the Protein S antibodies provided herein, or pharmaceutical compositions provided herein, wherein the condition is selected from the group consisting of: bleeding disorders, platelet disorders, trauma, bleeding resulting from a surgery or a medical procedure, and combinations thereof.

[0012a] In one aspect, the present disclosure refers to use of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating a condition in a subject in need thereof, wherein the condition is selected from the group consisting of: bleeding disorders, platelet disorders, trauma, and bleeding resulting from a surgery or a medical procedure.

[0013] In another example, provided herein is the use of any one of the Protein S antibodies or pharmaceutical compositions provided herein, for the treatment of a condition in a subject in need thereof. The condition may be selected from the group consisting of: bleeding disorders, platelet disorders, trauma, bleeding resulting from a surgery or a medical procedure, and combinations thereof.

[0014] In another example, any one of the Protein S antibodies or pharmaceutical compositions provided herein, may be used for the manufacture of a medicament for the treatment of a condition in a subject in need thereof. The condition may be selected from the group consisting of: bleeding disorders, platelet disorders, trauma, bleeding resulting from a surgery or a medical procedure, and combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] **FIG. 1** depicts a schematic diagram of Protein S showing the modular domains of Protein S.

[0016] **FIGS. 2A-2B** depict the results of screening assays performed to assess APC and TFPI cofactor activity, respectively, using control Protein S antibodies. In this and subsequent figures, ProS = Protein S; mAb = monoclonal antibody; pAb = polyclonal antibody; PRP = platelet rich plasma.

[0017] **FIGS. 3-4** depict peak thrombin levels generated by Protein S monoclonal antibodies, identified from hybridoma libraries, in the APC cofactor and TFPI cofactor screening assays, respectively.