210 pM, less than about 200 pM, less than about 190 pM, less than about 180 pM, less than about 170 pM, less than about 160 pM, less than about 150 pM, less than about 140 pM, less than about 130 pM, less than about 120 pM, less than about 110 pM, less than about 100 pM, less than about 95 pM, less than about 90 pM, less than about 85 pM, less than about 80 pM, less than about 75 pM, less than about 70 pM, less than about 65 pM, less than about 60 pM, less than about 55 pM, less than about 50 pM, less than about 45 pM, less than about 40 pM, less than about 35 pM, less than about 30 pM, less than about 25 pM, less than about 20 pM, less than about 15 pM, less than about 10 pM, less than about 9 pM, less than about 8 pM, less than about 7 pM, less than about 6 pM, less than about 5 pM, less than about 4 pM, or less than about 3 pM, as measured by surface plasmon resonance, *e.g.*, using the assay format as defined in Example 3 herein, or a substantially similar assay.

[0077] The present invention also includes anti-Activin A antibodies and antigen-binding fragments thereof that inhibit Activin A-mediated cellular signaling. For example, the present invention includes anti-Activin A antibodies that inhibit the activation of the SMAD complex signal transduction pathway via the binding of Activin A to Activin Type I or II receptors with an IC_{50} value of less than about 4 nM, as measured in a cell-based blocking bioassay, *e.g.*, using the assay format as defined in Example ~~5~~6 herein, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the present invention inhibit the activation of the SMAD complex signal transduction pathway via the binding of Activin A to Activin Type I or II receptors with an IC_{50} value of less than about 3nM, less than about 2 nM, less than about 1 nm, less than about 500 pM, less than about 250 pM, less than about 240 pM, less than about 230 pM, less than about 220 pM, less than about 210 pM, less than about 200 pM, less than about 190 pM, less than about 180 pM, less than about 170 pM, less than about 160 pM, less than about 150 pM, less than about 140 pM, less than about 130 pM, less than about 120 pM, less than about 110 pM, less than about 100 pM, less than about 95 pM, less than about 90 pM, less than about 85 pM, less than about 80 pM, less than about 75 pM, less than 70 pM, less than about 65 pM, less than about 60 pM, less than about 55 pM, less than about 50 pM, less than about 49 pM, less than about 48 pM, less than about 47 pM, less than about 46 pM, less than about 45 pM, less than about 44 pM, less than about 43 pM, less than about 42 pM, less than about 41 pM, less than about 40 pM, or less than about 39 pM, as measured in a cell-based blocking bioassay, *e.g.*, using the assay format as defined in Example ~~5~~6 herein, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the present invention inhibit the signaling activing of Activin B by interfering with the binding of Activin B to Activin Type I or II receptors with an IC_{50} value of less than about 50 nM, less than about 20 nM, less than about 10 nm, less than about 5 nM, or less than about 1 nM, , as measured in a cell-based blocking bioassay, *e.g.*, using the assay format as defined in Example ~~5~~6 herein, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the present invention inhibit the activation of the

SMAD complex signal transduction pathway via the binding of Activin AB to Activin Type I or II receptors with an IC_{50} value of less than about 500 pM, less than about 450 pM, less than about 440 pM, less than about 430 pM, less than about 420 pM, less than about 410 pM, less than about 400 pM, less than about 390 pM, less than about 380 pM, less than about 370 pM, less than about 360 pM, less than about 350 pM, less than about 340 pM, less than about 320 pM, less than about 310 pM, less than about 300 pM, less than about 290 pM, less than about 280 pM, less than about 270 pM, less than about 260 pM, less than about 250 pM, less than about 240 pM, less than about 230 pM, less than about 220 pM, less than about 210 pM, less than about 200 pM, less than about 190 pM, less than about 180 pM, less than about 170 pM, less than about 160 pM, less than about 150 pM, or less than about 140 pM, as measured in a cell-based blocking bioassay, *e.g.*, using the assay format as defined in Example ~~5~~6 herein, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the present invention inhibit the activation of the SMAD complex signal transduction pathway via the binding of Activin AC to Activin Type I or II receptors with an IC_{50} value of less than about 1 nM, less than about 900 pM, less than about 800 pM, less than about 750 pM, less than about 700 pM, less than about 650 pM, less than about 600 pM, or less than about 580 pM, as measured in a cell-based blocking bioassay, *e.g.*, using the assay format as defined in Example ~~5~~6 herein, or a substantially similar assay.

[0078] The antibodies of the present invention may possess one or more of the aforementioned biological characteristics, or any combinations thereof. Other biological characteristics of the antibodies of the present invention will be evident to a person of ordinary skill in the art from a review of the present disclosure including the working Examples herein.

Anti-Activin A Antibodies Comprising Fc Variants

[0079] According to certain embodiments of the present invention, anti-Activin A antibodies are provided comprising an Fc domain comprising one or more mutations which enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, the present invention includes anti-Activin A antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., H/L/R/S/P/Q or K) and/or 434 (*e.g.,* A, W, H, F or Y [N434A, N434W, N434H, N434F or N434Y]); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y)

[0083] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, *e.g.*, routine cross-blocking assay such as that described Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY), alanine scanning mutational analysis, peptide blots analysis (Reineke, Methods Mol Biol 248:443-463 (2004)), and peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer, Protein Science 9:487-496 (2000)). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water to allow hydrogen-deuterium exchange to occur at all residues except for the residues protected by the antibody (which remain deuterium-labeled). After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring, Analytical Biochemistry 267(2):252-259 (1999); Engen and Smith, Anal. Chem. 73:256A-265A (2001).

[0084] The present invention further includes anti-Activin A antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.*, H4H10423P, H4H10424P, H4H10426P, H4H10429P, H4H10430P, H4H10432P2, H4H10433P2, H4H10436P2, H4H10437P2, H4H10438P2, H4H10440P2, H4H10442P2, H4H10445P2, H4H10446P2, H4H10447P2, H4H10448P2, H4H10452P2, H4H10468P2, H2aM10965N, etc.). Likewise, the present invention also includes anti-Activin A antibodies that compete for binding to Activin A with any of the specific exemplary antibodies described herein (*e.g.*, H4H10423P, H4H10424P, H4H10426P, H4H10429P, H4H10430P, H4H10432P2, H4H10433P2, H4H10436P2, H4H10437P2, H4H10438P2, H4H10440P2, H4H10442P2, H4H10445P2, H4H10446P2, H4H10447P2, H4H10448P2, H4H10452P2, H4H10468P2, H2aM10965N, etc.). For example, the present invention includes anti-Activin A antibodies that cross-compete for binding to Activin A with one or more antibodies of "Bin 1" as defined in Example 45 herein (*e.g.*, H4H10423P, H4H10446P2, H4H10468P2 and H4H10442P2). The present invention also includes anti-Activin A antibodies that cross-compete for binding to Activin A with one or more antibodies of "Bin 2" as defined in Example 45 herein (*e.g.*, H4H10429, ~~H4H1430P~~ H4H10430P, H4H10432P2, H4H10436P2, and H4H10440P2).

[0085] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-Activin A antibody by using routine methods known in the art and exemplified herein. For example, to determine if a test antibody binds to the same epitope as a reference anti-Activin A antibody of the invention, the reference antibody is

competes with H4H10430P for binding to Activin A. Five antibodies, H4H10430P, H4H10429, H4H10432P2, H4H10436P2, and H4H10440P2, bi-directionally compete with each other for binding to Activin A, as well as with Control 1 and Control 3. Four of these five antibodies (*i.e.*, H4H10429, H4H10432P2, H4H10436P2, and H4H10440P2) do not cross compete with any other Activin A antibodies, whereas H4H10423P also cross-competes with H4H10430P, as noted above.

[00146] The results of this Example indicate that the anti-Activin A antibodies of the invention can be grouped into two distinct "bins" based on epitope binding characteristics: Bin 1 includes H4H10423P, H4H10446P2, H4H10468P2 and H4H10442P2. Bin 2 includes H4H10429, ~~H4H1430P~~ H4H10430P, H4H10432P2, H4H10436P2, and H4H10440P2. Further, one antibody from each bin, *i.e.*, H4H10423P and ~~H4H1430P~~H4H10430P, cross-compete with each other. The results of this Example suggest that the antibodies of Bin 1 bind to distinct regions on Activin A than the antibodies of Bin 2.

Example 6. Inhibition of Activin A-Mediated Receptor Activation and SMAD Complex Signaling with Anti-Activin A Antibodies

[00147] To further characterize anti-Activin A antibodies of the present invention, a bioassay was developed to detect the activation of the activin Type IIA and IIB receptors (ActRIIA and ActRIIB, respectively) and the subsequent phosphorylation and activation of an Activin Type I receptor. The interaction between ActRIIA and ActRIIB and activin leads to the induction of diverse cellular processes including growth regulation, metastatis of cancer cells and differentiation of embryonic stem cells (Tsuchida, K. *et al.*, Cell Commun Signal 7:15 (2009)). Phosphorylation and activation of the Type I receptor leads to phosphorylation of SMAD 2 and 3 proteins which form activated SMAD complexes leading to transcriptional regulation of genes.

[00148] To detect the activation of the SMAD complex signal transduction pathway via activin binding to activin Type II receptors, a human A204 rhabdomyosarcoma cell line (ATCC, # HTB-82) was transfected with a Smad 2/3-luciferase reporter plasmid (CAGAx12-Luc; Dennler, 1998) to create the A204/CAGAx12-Luc cell line. A204/CAGAx12-Luc cells were maintained in McCoy's 5A (Irvine Scientific, # 9090) supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin/glutamine and 250 µg/mL of G418. For the bioassay, A204/CAGAx12-Luc cells were seeded onto 96-well assay plates at 10,000 cells/well in low serum media, 0.5%FBS and OPTIMEM (Invitrogen, #31985-070), and incubated at 37°C and 5% CO_2 overnight. To determine the ligand dose response, Activin A (R&D Systems, #338-AC), Activin B (R&D Systems, #659-AB), Activin AB (R&D Systems, #1066-AB) and Activin AC (R&D Systems, #4879-AC/CF) were serially diluted at 1:3 from 100 to 0.002nM and added to cells starting along with a control containing no Activin. Activin A, Activin B, Activin AB, and Activin AC were observed to activate the A204/CAGAx12-Luc cell line with EC_{50} values of 99pM, 47pM, 19pM, and 4.4nM, respectively. To measure inhibition, antibodies were serially diluted at 1:3 starting from 100 to 0.002nM, 1000 to 0.02nM, or 300 to 0.005nM including control samples