tissue permeability and therefore can be used to increase the dispersion and delivery of therapeutic agents. Various hyaluronidases have been used therapeutically (*e.g.* Hydase™, Vitrase™ and Wydase™), typically as dispersing and spreading agents in combination with other therapeutic agents. Many of these are ovine or bovine forms, which can be immunogenic for treatment of humans. Improved compositions of hyaluronidases that can be used for treatment are needed.

SUMMARY

In a first aspect, there is provided an extended soluble PH20 hyaluronidase (esPH20) that is selected from among: a polypeptide that consists of the sequence of amino acids set forth as amino acid residues 36-491, 36-492, 36-493, 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107; or a polypeptide that contains amino acid substitutions in the sequence of amino acids set forth as amino acids 36-491, 36-492, 36-493, 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107, whereby the amino acid-substituted polypeptide consists of a sequence of amino acids that has at least 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity with the corresponding sequence of amino acids set forth as amino acids 36-491, 36-492, 36-493, 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107, wherein: the esPH20 polypeptide is soluble; and the esPH20 polypeptide exhibits catalytic activity at neutral pH.

In a second aspect, there is provided an esPH20 as defined above that is N-glycosylated.

In a third aspect, there is provided an esPH20 as defined above, wherein the esPH20 is N-glycosylated and comprises at least an N-acetylglucosamine moiety linked to each of at least three asparagine (N) residues.

In a fourth aspect, there is provided an esPH20 as defined above, wherein the three asparagine residues correspond to amino acid residues 235, 368 and 393 of SEQ ID NO: 107.

In a fifth aspect, there is provided an esPH20 as defined above that consists of the sequence of amino acids set forth as amino acids 36-491, 36-492, 36-493, 36-495, 36-496, 36-497, 36-498, 36-499 or 36-500 of SEQ ID NO: 107.

In a sixth aspect, there is provided an esPH20 as defined above that is modified by modification selected from among sialation, albumination, farnesylation, carboxylation, hydroxylation and phosphorylation.

In a seventh aspect, there is provided an esPH20 as defined above that is modified by a polymer.

In an eighth aspect, there is provided an esPH20 as defined above, wherein the polymer is dextran or PEG.

In a ninth aspect, there is provided an esPH20 as defined above that is not bifucosylated.

In a tenth aspect, there is provided a conjugate, comprising the esPH20 as defined above.

In an eleventh aspect, there is provided a conjugate as defined above, wherein the esPH20 is conjugated to a moiety selected from among a multimerization domain, toxin, detectable label or drug.

In a twelfth aspect, there is provided a conjugate as defined above, wherein the esPH20 is conjugated to an Fc domain.

In a thirteenth aspect, there is provided a conjugate as defined above, wherein the esPH20 is conjugated to a polymer.

In a fourteenth aspect, there is provided a conjugate as defined above, wherein the polymer is dextran or PEG.

In a fifteenth aspect, there is provided a conjugate as defined above, wherein the conjugated moiety is linked directly or via a linker to the C-terminus or N-terminus of the esPH20.

In a sixteenth aspect, there is provided a nucleic acid molecule encoding the esPH20 as defined above, wherein the nucleic acid molecule does not encode a full-length PH20 but encodes an esPH20.

In a seventeenth aspect, there is provided an expression vector, comprising a polynucleotide that consists of the sequence of nucleotides encoding a esPH20 as defined above each including or immediately followed by a stop codon operatively inserted into the vector for expression of the esPH20 as defined above.

In an eighteenth aspect, there is provided a vector, comprising the nucleic acid as defined above.

In a nineteenth aspect, there is provided a cell, comprising a vector as defined above or a nucleic acid molecule as defined above.

In a twentieth aspect, there is provided the cell as defined above that is a CHO cell.

In a twenty-first aspect, there is provided a composition, comprising an esPH20 polypeptide as defined above.

In a twenty-second aspect, there is provided a composition, comprising a plurality of esPH20 polypeptides as defined above.

In a twenty-third aspect, there is provided a composition as defined above, that is a pharmaceutical composition.

In a twenty-fourth aspect, there is provided a composition as defined above, comprising an additional therapeutic agent.

In a twenty-fifth aspect, there is provided the use of an esPH20 as defined above or a composition as defined above in the formulation of a medicament for treating a hyaluronan-associated disease or condition.

In a twenty-sixth aspect, there is provided a pharmaceutical composition, comprising a polypeptide as defined above for treating a hyaluronan-associated disease or condition.

In a twenty-seventh aspect, there is provided a pharmaceutical composition as defined above for treating a hyaluronan-associated disease or condition.

In a twenty-eighth aspect, there is provided the use of an esPH20 as defined above or a composition as defined above for the formulation of a medicament for treating an excess of glycosaminoglycans; for treating a tumor; for treating glycosaminoglycan accumulation in the brain; for treating a cardiovascular disorder; for treating an ophthalmic disorder; for treating pulmonary disease; for increasing penetration of chemotherapeutic agents into solid tumors; for treating cellulite; for treating a proliferative disorder; or for increasing bioavailability of drugs and other therapeutic agents.

In a twenty-ninth aspect, there is provided a pharmaceutical composition, comprising a polypeptide as defined above, for use in treating an excess of glycosaminoglycans; for treating a tumor; for treating glycosaminoglycan accumulation in the brain; for treating a cardiovascular disorder; for treating an ophthalmic disorder; for treating pulmonary disease; for increasing penetration of chemotherapeutic agents into solid tumors; for treating cellulite; for treating a proliferative disorder; or for increasing bioavailability of drugs and other therapeutic agents.

In a thirtieth aspect, there is provided a pharmaceutical composition as defined above for use in treating an excess of glycosaminoglycans; for treating a tumor; for treating glycosaminoglycan accumulation in the brain; for treating a cardiovascular disorder; for treating an ophthalmic disorder; for treating pulmonary disease; for increasing penetration of chemotherapeutic agents into solid tumors; for treating cellulite; for treating a proliferative disorder; or for increasing bioavailability of drugs and other therapeutic agents.

Provided herein are soluble PH20 polypeptides, including extended soluble PH20 (esPH20) polypeptides, and compositions. The PH20 polypeptides provided herein are soluble proteins that are truncated at the C-terminus and include those that lack all of the GPI-anchor attachment signal sequence (e.g. are truncated at amino acid positions 450 to 490). Soluble PH20 polypeptides also include extended soluble PH20 polypeptides that retain one or more residues located in the GPI-anchor attachment signal sequence of the corresponding full length wild-type PH20 polypeptide. Also provided herein are other modified PH20 polypeptides that contain C-terminal truncations. Partially deglycosylated forms of any of the polypeptides also are provided. Also provided are methods of treatment using the PH20 polypeptides provided herein.

Provided herein are isolated substantially purified extended soluble PH20 (esPH20) hyaluronidases, that can be N-glycosylated or N-partially glycosylated. In some examples, the N-partially glycosylated esPH20 polypeptide contains at least an N-acetylglucosamine moiety linked to each of at least two N-linked moieties, such as, for example, amino acid residues 368 and 393 of SEQ ID NO:107 or residues corresponding to amino acid residues 368 and 393 of SEQ ID NO:107. In some aspects, the N-partially glycosylated esPH20 polypeptide contains at least two N-acetylglucosamine moieties linked to each of at least two N-linked moieties. The N-partially glycosylated esPH20 polypeptide provided herein also can contain a branched sugar.

Provided herein are esPH20 polypeptides that have the sequence of amino acids set forth in any of SEQ ID NOS: 60-63 and 102-104 or an allelic or species variant thereof. Also provided are esPH20 polypeptide variants having at least 40%,