DOWNLOAD PDF SERINE PROTEASES AND THEIR SERPIN INHIBITORS IN THE NERVOUS SYSTEM

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serine proteases and their inhibitors in the nervous system When we decided, in the Fall of , to hold a NATO Advanced Research Workshop on the topic of the possible roles and regulation of serine proteases and.

Substrate specificity[edit] Serine proteases are characterised by a distinctive structure, consisting of two beta-barrel domains that converge at the catalytic active site. These enzymes can be further categorised based on their substrate specificity as either trypsin-like, chymotrypsin-like or elastase-like. Chymotrypsin-like[edit] The S1 pocket of chymotrypsin-like enzymes is more hydrophobic than in trypsin-like proteases. This results in a specificity for medium to large sized hydrophobic residues, such as tyrosine , phenylalanine and tryptophan. Thrombin-like[edit] These include thrombin , tissue activating plasminogen and plasmin. Elastase-like[edit] Elastase-like proteases have a much smaller S1 cleft than either trypsin- or chymotrypsin-like proteases. Consequently, residues such as alanine , glycine and valine tend to be preferred. Subtilisin-like[edit] Subtilisin is a serine protease in prokaryotes. Subtilisin is evolutionarily unrelated to the chymotrypsin-clan, but shares the same catalytic mechanism utilising a catalytic triad , to create a nucleophilic serine. This is the classic example used to illustrate convergent evolution , since the same mechanism evolved twice independently during evolution. The triad is located in the active site of the enzyme, where catalysis occurs, and is preserved in all superfamilies of serine protease enzymes. The triad is a coordinated structure consisting of three amino acids: His 57, Ser hence the name "serine protease" and Asp These three key amino acids each play an essential role in the cleaving ability of the proteases. While the amino acid members of the triad are located far from one another on the sequence of the protein, due to folding, they will be very close to one another in the heart of the enzyme. The particular geometry of the triad members are highly characteristic to their specific function: The catalysis of the peptide cleavage can be seen as a ping-pong catalysis, in which a substrate binds in this case, the polypeptide being cleaved , a product is released the N-terminus "half" of the peptide , another substrate binds in this case, water , and another product is released the C-terminus "half" of the peptide. Each amino acid in the triad performs a specific task in this process: The serine has an -OH group that is able to act as a nucleophile , attacking the carbonyl carbon of the scissile peptide bond of the substrate. A pair of electrons on the histidine nitrogen has the ability to accept the hydrogen from the serine -OH group, thus coordinating the attack of the peptide bond. The carboxyl group on the aspartic acid in turn hydrogen bonds with the histidine , making the nitrogen atom mentioned above much more electronegative. The whole reaction can be summarized as follows: The polypeptide substrate binds to the surface of the serine protease enzyme such that the scissile bond is inserted into the active site of the enzyme, with the carbonyl carbon of this bond positioned near the nucleophilic serine. The serine -OH attacks the carbonyl carbon, and the nitrogen of the histidine accepts the hydrogen from the -OH of the [serine] and a pair of electrons from the double bond of the carbonyl oxygen moves to the oxygen. As a result, a tetrahedral intermediate is generated. The bond joining the nitrogen and the carbon in the peptide bond is now broken. The covalent electrons creating this bond move to attack the hydrogen of the histidine , breaking the connection. The electrons that previously moved from the carbonyl oxygen double bond move back from the negative oxygen to recreate the bond, generating an acyl-enzyme intermediate. Now, water comes into the reaction. Water replaces the N-terminus of the cleaved peptide, and attacks the carbonyl carbon. Once again, the electrons from the double bond move to the oxygen making it negative, as the bond between the oxygen of the water and the carbon is formed. This is coordinated by the nitrogen of the histidine , which accepts a proton from the water. Overall, this generates another tetrahedral intermediate. In a final reaction, the bond formed in the first step between the serine and the carbonyl carbon moves to attack the hydrogen that the histidine just acquired. The now electron-deficient carbonyl carbon re-forms the double bond with the oxygen. As a result, the C-terminus of the peptide is now ejected. Additional stabilizing effects[edit] It was discovered that additional amino acids of the protease, Gly and Ser , are involved in creating what is called an oxyanion hole. Both Gly and Ser can donate backbone

hydrogens for hydrogen bonding. When the tetrahedral intermediate of step 1 and step 3 are generated, the negative oxygen ion, having accepted the electrons from the carbonyl double bond, fits perfectly into the oxyanion hole. In effect, serine proteases preferentially bind the transition state and the overall structure is favored, lowering the activation energy of the reaction. This "preferential binding" is responsible for much of the catalytic efficiency of the enzyme. Regulation of Serine Protease activity[edit] Host organisms must ensure that the activity of serine proteases is adequately regulated. This is achieved by a requirement for initial protease activation, and the secretion of inhibitors. Zymogen activation[edit] Zymogens are the usually inactive precursors of an enzyme. If the digestive enzymes were active when synthesized, they would immediately start chewing up the synthesizing organs and tissues. Acute pancreatitis is such a condition, in which there is premature activation of the digestive enzymes in the pancreas, resulting in self-digestion autolysis. It also complicates postmortem investigations , as the pancreas often digests itself before it can be assessed visually. Zymogens are large, inactive structures, which have the ability to break apart or change into the smaller activated enzymes. The difference between zymogens and the activated enzymes lies in the fact that the active site for catalysis of the zymogens is distorted. As a result, the substrate polypeptide cannot bind effectively, and proteolysis does not occur. Only after activation, during which the conformation and structure of the zymogen change and the active site is opened, can proteolysis occur.

Chapter 2 : Serine protease - Wikipedia

Serine Proteases and Their Serpin Inhibitors in the Nervous System Regulation in Development and in Degenerative and Malignant Disease. Editors: Festoff, Barry W. (Ed.).

To determine the potential activity of MSP in CNS demyelination, we examined its expression in multiple sclerosis lesions and in two animal models of multiple sclerosis: High levels of MSP were present within infiltrating mononuclear cells, including macrophages and T cells, which characteristically fill sites of demyelination, both in multiple sclerosis lesions and in animal models of this disease. Transfection of oligodendrocyte progenitors with an MSPâ€"green fluorescent protein construct produced similar changes in oligodendrocyte process number. We further demonstrate that myelin basic protein, and to a lesser extent myelin oligodendrocyte glycoprotein, can serve as MSP substrates. These studies support the hypothesis that excess MSP, as is present in inflammatory CNS lesions, promotes demyelination. Established or proposed roles of serine proteases and their endogenous serpin inhibitors include: These events are mediated, in part, by the ability of serine proteases to cleave, thereby activating growth factor precursor proteins, to degrade components of the extracellular matrix, and to bind to cell surface receptors, activating intracellular signalling cascades. The enzymatic activity of serine proteases is tightly regulated, afforded in part by a series of specific endogenous serpin inhibitors. Imbalances between proteases and their inhibitors, due to injury or disease, have been shown to result in CNS pathogenesis, including neuronal degeneration Tsirka et al. Whereas only three kallikrein genes were originally thought to exist in humans, 14 members have now been identified, aligned on chromosome 19q Yousef and Diamandis, While several of these newly identified genes have been reported to be expressed in brain, much still needs to be learned regarding their normal physiological roles, and their potential contributions to CNS pathogenesis. We previously showed that MSP is abundantly expressed by neurones and in a subpopulation of white matter glia in the human and rodent CNS Scarisbrick et al. Remarkably, within normal white matter, MSP expression is almost exclusively associated with oligodendroglia Scarisbrick et al. Given the abundant expression of MSP in oligodendroglia of the adult CNS, and regulation by injury, in this study we set out to determine its potential involvement in CNS demyelinating disease. Collectively, these studies indicate that MSP is a multifunctional serine protease, which may participate in multiple effector pathways governing demyelination of the CNS. All cases underwent detailed neuropathological examination and were screened for white matter demyelinating lesions. Demyelinating activity was classified according to recently established criteria Lassmann et al. Active demyelinating lesions were diffusely infiltrated by macrophages containing myelin proteins as markers of recent and ongoing myelin phagocytosis. Inactive demyelinated lesions were completely demyelinated without signs of remyelination. Clinical signs of EAE developed between 19 and 23 days after immunization. In all cases, control for the specificity of immunostaining included staining as above with the omission of primary antibody. Hybridization was performed as described previously Scarisbrick et al. The digested sample was resolved on Oligodendrocyte cell culture systems Two oligodendrocyte culture systems were used; purified oligodendrocyte progenitors and the bipotential CG4 oligodendrocyte cell line Louis et al. As above, cells were then allowed to differentiate for a further 72 h before analysis. To distinguish between cell surface or substrate effects, in a third paradigm, CG4 O2A cells were treated in one of three ways: Cells were differentiated for a further 24 h prior to analysis. On average, cells were counted per culture condition in each experiment. All experiments were performed in triplicate and repeated at least twice using independent cell culture preparations. Regulated expression of MSP in animal models of multiple sclerosis To profile the activity of MSP in CNS demyelination, we have examined its expression in two previously characterized animal models of multiple sclerosis. We previously demonstrated in rat and human that MSP in normal white matter is largely confined to oligodendroglia Fig. While observed infrequently in the normal cord Scarisbrick et al. Notably, neither of these treatments had a significant effect on the total number of cells stained by the

nuclear stain or bisbenzamide, or the percentage of those immunoreactive for O4 Fig.

Chapter 3 : SERPINI1 - Wikipedia

Serine Proteases and Their Serpin Inhibitors in the Nervous System: Regulation in Development and in Degenerative and Malignant Disease (Nato Science Series A:) Softcover reprint of the original 1st ed. Edition.

Three major groups of protease inhibitors are secreted into saliva: Kunitz inhibitors, serpins, and cystatins. Serpins and cystatins are also anti-hemostatic effectors, but intriguingly, from the translational perspective, also act as pluripotent modulators of the host immune system. Here we focus especially on this latter aspect of protease inhibition by ticks and describe the current knowledge and data on secreted salivary serpins and cystatins and their role in tick-host-pathogen interaction triad. We also discuss the potential therapeutic use of tick protease inhibitors. Serpins and Cystatins as Homeostatic Regulators Proteases also proteinases or peptidases are ubiquitous enzymes that cleave proteins to smaller peptides and amino acids. Proteases participate in a range of physiological processes including extracellular digestion, protein degradation, and tissue development Rawlings and Salvesen, Relevant to this review, however, is the fact that many proteases, in particular highly substrate-specific endopeptidases, mediate defense and homeostatic processes in both vertebrates and invertebrates. Proteolytic pathways rely on the precise and tightly regulated activation and inhibition of these endopeptidases. As a result of this evolutionary need, many crucial pathophysiological processes are regulated via proteolytic cascades, with notable examples being coagulation of plasma or haemolymph in arthropods , bacterial wall perforation with complement, or melanization in arthropods Amara et al. Each step involves proteolytic activation of another downstream protease, and all proteases in such cascades usually have their own endogenous inhibitors that balance the system. The role of arthropod protease inhibitors in the defense is supported by the fact that the expression of serpins and cystatins in Ixodes scapularis nymphs was attenuated upon infection with Anaplasma phagocytophilum, as seen in the transcriptomic data Ayllon et al. On the other hand, the expression of protease inhibitors in salivary glands and midguts of adult females differed among individual inhibitors, i. Similar data were collected from Ixodes ricinus infected with Bartonella henselae Liu et al. Therefore, precise involvement of every individual inhibitor in tick infection would have to be evaluated experimentally. Other intracellular and extracellular processes, such as cytokine activation, phagocytosis, intracellular signaling, and antigen processing, are also dependent on proteolysis Muller et al. Serpins and cystatins are the two main superfamilies of endogenous serine and cysteine protease inhibitors involved in the regulation of these processes. It is therefore unsurprising that both groups of inhibitors are well represented in parasites and are important in their interactions with hosts Schwarz et al. In order to obtain a blood meal, ticks secrete hundreds of different pharmacoactive molecules into the host via their saliva. These molecules have anti-hemostatic, anti-inflammatory, anti-complement and immunomodulatory properties and their function is to overcome or evade host defense mechanisms including immune response Brossard and Wikel, ; Chmelar et al. Moreover, tick saliva and also several salivary compounds were found to facilitate and enhance the establishment of tick-borne pathogens in the host Anguita et al. Inhibitors of proteases represent the most prominent protein families in tick salivary secretion that are responsible for alteration of many different host defense pathways. Serine Protease Inhibitors in Ticks Four groups of serine protease inhibitors have been described in ticks: Kunitz domain inhibitors, Kazal domain inhibitors, trypsin inhibitor-like cysteine rich domain TIL inhibitors, and serpins. Inhibitors with 1â€”7 Kunitz domains mostly act as anti-hemostatic proteins and form a large multigenic family of secreted salivary proteins in ticks that have probably played a crucial role in the development of tick hematophagy Corral-Rodriguez et al. Moreover, single Kunitz-domain inhibitors in other organisms are involved in ion channel blockade and may play a similar role in ticks Frazao et al. Kazal domain inhibitors are described in hematophagous insects such as mosquitoes and triatomine bugs Rimphanitchayakit and Tassanakajon, , but they are only rarely reported in ticks, in which their function is still unknown Zhou et al. TIL-domain inhibitors represent an interesting group of small inhibitors with a conserved

5-disulphide bridge structure that were first reported in Apis melifera Bania et al. The sequences of over 80 TIL-domain inhibitors have been found in arthropod genomes Zeng et al. Serpins Serpins form the largest superfamily of protease inhibitors, and they are ubiquitously distributed in nature including viruses and prokaryotes. With over 1, members, serpins are the most studied protease inhibitors Law et al. For example, there are 29 inhibitory and seven non-inhibitory serpins in humans and 60 functional serpin genes in mice Heit et al. Angiotensinogen is a non-inhibitory serpin that is proteolytically activated by renin into several oligopeptides angiotensins that regulate vasoconstriction and blood pressure Lu et al. Inhibitory serpins have very diverse functions depending on their specificity, but their importance is highlighted by the serpinopathiesâ€"diseases caused by serpin dysfunction or deficiency Belorgey et al. Emphysema, cirrhosis, angioedema, hypertension, and even familial dementia are caused at least in part by serpin dysfunction Kim et al. Arthropod serpins have mostly immunological and hemostatic functions. Serpins have been shown to regulate haemolymph coagulation, are involved in phenoloxidase system activation in insects, and regulate an immune toll pathway in haemolymph Kanost, ; Gulley et al. Indeed, several insect serpins act as anti-coagulants, anti-complement proteins and immunosuppressors Stark and James, , ; Colinet et al. Serpins are abundant in ticks, and one of their functions is to modulate host immune system. Recent advances in this area have been facilitated by the publication of I. In , Mulenga and colleagues found 45 serpins in the genome of I. Two years earlier, the same group described 17 serpins Lospins in Amblyomma americanum Mulenga et al. This number was, however, substantially broadened by the combination of several approaches up to approximately serpins Karim and Ribeiro, ; Porter et al. In the work of Porter and colleagues Porter et al. The conservation seems to be higher in serpins with basic or polar uncharged amino acid residues at P1 site Porter et al. Other 32 serpin transcripts from the Amblyomma genus were found in Amblyomma maculatum Karim et al. Two groups described 18 and 22 serpins in R. Another recent publication described 10 different serpin transcripts in the sialotranscriptome of the tick Hyalomma excavatum Ribeiro et al. Despite high number of identified transcripts, only small portion was characterized functionally. Tick Serpins with Known Function To date, almost 20 tick serpins from different tick species have been functionally validated by in vitro assays, in vivo experimental models, vaccination and by RNA interference RNAi experiments Table 1. These are detailed below. Tick serpins with known function. Recombinant AamS6 inhibited the serine proteases trypsin, chymotrypsin, elastase, and chymase and the cysteine protease papain in a dose-dependent manner Chalaire et al. AamS6 also reduced platelet aggregation and delayed plasma clotting time, suggesting that this serpin facilitates blood feeding by ticks Mulenga et al. The complement activation pathway, however, was not affected Mulenga et al. AAS19 inhibited thrombinâ€"but not ADPâ€"and cathepsin G-activated platelet aggregation and delayed clotting in re-calcification and thrombin time assays Kim et al. In rabbits, immunized with AAS19, tick feeding was faster, but smaller blood volumes were ingested, and tick ability to lay eggs was impaired Kim et al. Recombinant HLS1 displayed anticoagulant activity, and nymph and adult tick feeding on immunized rabbits resulted in Antibodies raised against tick saliva did not react with recombinant HSL1, suggesting that the serpin was not secreted Sugino et al. Moreover, HLS1 expression was detected in the midgut rather than the salivary glands, and HLS1 was therefore considered a concealed antigen, similar to the first commercially used anti-tick vaccine based on the Bm86 tick protein Willadsen et al. HLS1 does not contain a signal peptide. Therefore, it is likely that HLS1 is not a secreted protein playing an immunomodulatory or anti-hemostatic role in the host during tick feeding. A second serpin from H. HLS2 prolonged the coagulation time in a dose-dependent manner Imamura et al. This might be explained by better accessibility and inactivation of extracellular HLS2 in the haemolymph by antibodies from the ingested blood of immunized animals. Ipis-1 Ixodes persulcatus To date, Ipis-1 is the only characterized salivary serpin from tick I. Ipis-1 transcripts were detected only in salivary glands of ticks at same level throughout all phases of feeding. Authors suggest that Ipis-1 could inhibit T cells function by direct interaction with this cell population Toyomane et al. Iris displayed several notable and important features. First, Iris was noted to inhibit T cell and splenocyte proliferation and altered peripheral blood mononuclear cell PBMC -derived

cytokine levels Leboulle et al. Second, Iris showed anti-hemostatic properties including suppression of coagulation and fibrinolysis Prevot et al. Interestingly, these activities were independent on the protease inhibitory function of Iris. Of note, Iris, together with HLS1 and several other proteins, belongs to a group of serpins in Ixodes spp. However, Iris has been detected in tick saliva using a polyclonal serum raised against recombinant protein Leboulle et al. This contradictory observation might be explained by cross-reactivity with another secreted serpin or by the action of another, non-classical secretory mechanism Nickel, Nevertheless, Iris represents a pleiotropic protein that affects multiple processes simultaneously via independent mechanisms. IRS-2 has tryptophan in its P1 site, confirmed by its resolved crystal structure Kovarova et al. IRS-2 displayed inhibitory specificity against mast cell chymase and cathepsin G, two proteases involved in inflammatory responses Chmelar et al. IRS-2 also inhibited platelet aggregation induced by cathepsin G but not other inducers such as collagen or arachidonic acid derivatives Chmelar et al. Furthermore, IxscS-1E1 inhibited adenosine diphosphate- and thrombin-activated platelet aggregation and delayed plasma clotting time, suggesting an anti-hemostatic role Ibelli et al. IxscS-1E1 had no effect on the classical complement activation pathway Ibelli et al. This finding is, however, consistent with their predicted intracellular location Imamura et al. However, no significant protective effect against infection with T. Both inhibited chymotrypsin, and RHS-1 also inhibited thrombin Yu et al. Consistent with their inhibitory activity, only RHS-1 exhibited anticoagulation activity based on the activated partial thrombin time assay Yu et al. Only RHS-1 seems to be secreted into the saliva and the host, as only RHS-1 was detected by serum from rabbits that were exposed to ticks and only RHS-1 possesses a signal peptide sequence Yu et al. Nevertheless, RNAi of both serpins negatively affected the attachment rate after 24 h and decreased the engorgement rate Yu et al. RmS-3, 6, 15, 17 R. Tirloni and colleagues subsequently confirmed this specificity albeit with much lower inhibitory activity , tested more proteases, and found the highest inhibitory activity against chymase and cathepsin G Tirloni et al. RmS-3 is likely to be secreted into the saliva and the host as evidenced by differential antibody responses of tick-resistant and tick-susceptible cattle Rodriguez-Valle et al. RmS-3 is expressed in nymphs and in the salivary glands of adult ticks, data on RmS-3 transcription in ovaries differ between the two studies Tirloni et al. Capillary feeding of ticks with a RmS-3 antibody reduced tick reproductive capacity Rodriguez-Valle et al. In addition to RmS-3, three other recombinant R. RmS-6 inhibited factor Xa, factor XIa and plasmin, suggesting an anticoagulant function, while RmS showed weaker inhibitory activity against chymotrypsin, cathepsin G, trypsin, and plasmin Tirloni et al. Interestingly, RmS-3, -6, and from R. RmS was identified as a thrombin inhibitor and, together with RmS, delayed plasma clotting in a re-calcification time assay Tirloni et al. Moreover, RmS is an immunogen, as the infestation of cattle with R. Cystatins Cystatins form a superfamily of tight-binding reversible inhibitors of papain-like cysteine proteases and legumains and, similar to serpins, they are present in all organisms including prokaryotes Kordis and Turk, Cystatins regulate many physiological processes including immunity-related mechanisms such as antigen presentation, phagocytosis, and cytokine expression Zavasnik-Bergant,

Chapter 4 : Activity of a newly identified serine protease in CNS demyelination | Brain | Oxford Academic

Serine proteases and their serpin inhibitors in the nervous system: regulation in development and in degenerative and malignant disease.

History[edit] Protease inhibitory activity in blood plasma was first reported in the late s, [10] but it was not until the s that the serpins antithrombin and alpha 1-antitrypsin were isolated. These proteases possess a nucleophilic serine residue in a catalytic triad in their active site. Examples include thrombin , trypsin , and human neutrophil elastase. These enzymes differ from serineproteases in that they use a nucleophilic cysteine residue, rather than a serine , in their active site. For example, extracellular serpins regulate the proteolytic cascades central to blood clotting antithrombin , the inflammatory and immune responses antitrypsin, antichymotrypsin , and C1-inhibitor and tissue remodelling PAI The protease targets of intracellular inhibitory serpins have been difficult to identify, since many of these molecules appear to perform overlapping roles. Further, many human serpins lack precise functional equivalents in model organisms such as the mouse. Nevertheless, an important function of intracellular serpins may be to protect against the inappropriate activity of proteases inside the cell. In doing so, Serpin B9 may protect against inadvertent release of granzyme B and premature or unwanted activation of cell death pathways. The cowpox viral serpin CrmA cytokine response modifier A is used in order to avoid inflammatory and apoptotic responses of infected host cells. Thyroxine-binding globulin and transcortin transport the hormones thyroxine and cortisol , respectively. Its exact function is unknown, but it is thought to be a storage protein for the developing foetus. The reactive centre loop RCL, blue exists in a dynamic equilibrium between the fully exposed conformation left and a conformation where it is partially inserted into the breach of the A-sheet right. The RCL forms the initial interaction with the target protease in inhibitory molecules. Structures have been solved showing the RCL either fully exposed or partially inserted into the A-sheet, and serpins are thought to be in dynamic equilibrium between these two states. Structural biology has therefore played a central role in the understanding of serpin function and biology. Instead, serpins use an unusual conformational change , which disrupts the structure of the protease and prevents it from completing catalysis. This converts the serpin from a stressed state, to a lower-energy relaxed state S to R transition. Initially, the catalytic residue of the active site triad performs a nucleophilic attack on the peptide bond of the substrate. This releases the new N-terminus and forms a covalent ester -bond between the enzyme and the substrate. For standard substrates , the ester bond is hydrolysed and the new C-terminus is released to complete catalysis. However, when a serpin is cleaved by a protease, it rapidly undergoes the S to R transition before the acyl-enzyme intermediate is hydrolysed. Since the RCL is still covalently attached to the protease via the ester bond, the S to R transition pulls protease from the top to the bottom of the serpin and distorts the catalytic triad. The distorted protease can only hydrolyse the acyl enzyme intermediate extremely slowly and so the protease remains covalently attached for days to weeks. The serpin white first binds a protease grey with the exposed reactive centre loop blue. When this loop is cleaved by the protease, it rapidly inserts into the A-sheet light blue , deforming and inhibiting the protease. First, the substrate blue is attacked by the cysteine or serine of the catalytic triad red to form an acyl-enzyme intermediate. For typical substrates, the intermediate is resolved by hydrolysis by water. However, when the reactive centre loop RCL of a serpin is attacked, the conformational change blue arrow pulls the catalytic triad out of position, preventing it from completing catalysis. The serpin antithrombin has an RCL blue where the P1 arginine blue sticks points inwards, preventing protease binding. Binding of heparin green sticks causes the P1 arginine residue to flip to an exposed position. The target protease grey then binds to both the exposed P1 arginine as well as the heparin. The serpin then activates and heparin is released. The partially inserted conformation is important because co-factors are able to conformationally switch certain partially inserted serpins into a fully expelled form. The archetypal example of this situation is antithrombin, which circulates in plasma in a partially inserted relatively inactive state. The primary specificity determining residue the P1

arginine points toward the body of the serpin and is unavailable to the protease. Upon binding a high-affinity pentasaccharide sequence within long-chain heparin , antithrombin undergoes a conformational change, RCL expulsion, and exposure of the P1 arginine. The heparin pentasaccharide-bound form of antithrombin is, thus, a more effective inhibitor of thrombin and factor Xa. Heparin, therefore, also acts as a template for binding of both protease and serpin, further dramatically accelerating the interaction between the two parties. After the initial interaction, the final serpin complex is formed and the heparin moiety is released. This interaction is physiologically important. For example, after injury to the blood vessel wall, heparin is exposed, and antithrombin is activated to control the clotting response. Understanding of the molecular basis of this interaction enabled the development of Fondaparinux , a synthetic form of Heparin pentasaccharide used as an anti-clotting drug. The serpin PAI-1 remains in the active conformation when bound to vitronectin green. However, in the absence of vitronectin, PAI-1 can change to the inactive latent state. Latent serpins are unable to interact with proteases and so are no longer protease inhibitors. The conformational change to latency is not exactly the same as the S to R transition of a cleaved serpin. Although PAI-1 is produced in the inhibitory S conformation, it "auto-inactivates" by changing to the latent state unless it is bound to the cofactor vitronectin. Disruption of interactions made by the N-terminal region results in spontaneous conformational change of this serpin to the latent conformation. For example, the native S form of thyroxine-binding globulin has high affinity for thyroxine, whereas the cleaved R form has low affinity. Similarly, transcortin has higher affinity for cortisol when in its native S state, than its cleaved R state. Thus, in these serpins, RCL cleavage and the S to R transition has been commandeered to allow for ligand release, rather than protease inhibition. In these cases, a serpin that has formed a complex with its target protease, is then recognised by a receptor. The binding event then leads to downstream signalling by the receptor. For extracellular serpins, the final serpin-enzyme complexes are rapidly cleared from circulation. One mechanism by which this occurs in mammals is via the low-density lipoprotein receptor-related protein LRP , which binds to inhibitory complexes made by antithrombin, PA, and neuroserpin, causing cellular uptake. Mutations that change the activity, specificity or aggregation properties of serpins all affect how they function. The majority of serpin-related diseases are the result of serpin polymerisation into aggregates, though several other types of disease-linked mutations also occur. Four residues of the RCL blue; disordered region as dashed line are inserted into the top of the A-sheet. Since a serpin can only make this conformational change once, the resulting misfired serpin is inactive and unable to properly control its target protease. For example, the disease-linked antithrombin variants wibble and wobble, [62] both promote formation of the latent state. The polymers are therefore hyperstable to temperature and unable to inhibit proteases. Serpinopathies therefore cause pathologies similarly to other proteopathies e. Second, the hyperstable polymers themselves clog up the endoplasmic reticulum of cells that synthesize serpins, eventually resulting in cell death and tissue damage. In the case of antitrypsin deficiency, antitrypsin polymers cause the death of liver cells, sometimes resulting in liver damage and cirrhosis. Within the cell, serpin polymers are slowly removed via degradation in the endoplasmic reticulum. In the dimer of antithrombin , the RCL and part of the A-sheet incorporates into the A-sheet of another serpin molecule. Most intracellular serpins belong to a single phylogenetic clade, whether they come from plants or animals, indicating that the intracellular and extracellular serpins may have diverged before the plants and animals. The S to R conformational change has also been adapted by some binding serpins to regulate affinity for their targets.

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Chapter 5 : About us - Serpinx

Get this from a library! Serine Proteases and Their Serpin Inhibitors in the Nervous System: Regulation in Development and in Degenerative and Malignant Disease.

Advanced Search Abstract Cumulative evidence has shown that a delicate balance between serine proteases and their inhibitors is crucial for normal functioning of several biological pathways. The importance of proteases and their inhibitors is well documented in several human diseases. Among them, the best documented are hemophilia B, a genetic deficiency of the serine protease coagulation factor IX and serpinophathies. Alphaantitrypsin deficiency MIM , is associated with early-onset emphysema and liver disease, while hereditary angioedema HANE; MIM is caused by mutations in the C1 inhibitor, a serpin involved in the regulation of the complement cascade. Recently, two human genetic diseases of the central nervous system have been related to mutations in components of extracellular proteolytic systems. Here, we review the recent advances in this field. Serine proteases act sequence-specifically and are usually synthesized and secreted as inactive proenzymes called zymogens further activated by proteolysis. The best characterized members of this family play a role in intestinal digestion, blood coagulation and fibrinolysis i. In addition, several studies have pointed out the role of serine proteases in the central nervous system CNS 1. Indeed, during neural development, serine proteases contribute to cell migration, axon outgrowth and synapse elimination. In adult, they play a role in neuropeptide processing, regulation of neuronal survival and structural plasticity associated with learning and memory processes. For instance, urokinase-plasminogen activator uPA and tissue type-plasminogen activator tPA convert plasminogen into active plasmin which in turn remodels synaptic connections via the degradation of an extracellular matrix component, laminin 2. Finally, neuropsin, a serine protease exclusively expressed in the central nervous system, cleaves the Ig superfamily cell adhesion molecule L1, which is located in the presynaptic membrane and plays an important role in the maintenance of long-term potentiation LTP 4. The serine protease inhibitors of the serpin family act by binding to the active site of their target protease s 8. This physiological inhibitory mechanism involves docking of the serpin to the target protease, cleavage of the reactive center loop, and rapid translocation of the protease to the opposite pole of the serpin 8. Protease nexin-1 PN-1 , the first identified neural serpin, is expressed in glia and neurons 9 â€” It is a potent inhibitor of thrombin, but it can also inhibit tPA. Mice overexpressing PN-1 in neurons show increased LTP in the CA1 field of hippocampal slices and develop progressive disturbances of motor behavior and sensorimotor integration, while PNdeficient mice have decreased hippocampal LTP 12 , Both overexpression and lack of PN-1 cause epileptic activity in vivo and in vitro. Neuroserpin, the second neural serpin, has been recognized as an axonally secreted member of the serpin superfamily Searches for target proteases 15 , 16 revealed strong inhibition of tPA and uPA and to a somewhat lesser degree of plasmin, but no action against thrombin was noted. This raises the question of whether tPA is really the cognate target protease of neuroserpin. Plasminogen activator inhibitor 1 PAI-1 , the cognate inhibitor of tPA in plasma, is not normally expressed in the brain, although it may be induced after exitotoxic injury Therefore, it is possible that the cognate target of neuroserpin may be another, as yet unidentified, serine protease. Likewise, the activity of tPA activity in the brain might be controlled by an inhibitor other than neuroserpin. The severity of mental retardation is commonly classified on the basis of intelligence quotient IQ although other criteria have also been used. The causes of MR are diverse and include environmental factors, teratogens, chromosomal anomalies and metabolic diseases impairing neuronal function. MRs of genetic origin include metabolic diseases impairing neuronal function in a non-specific manner and conditions which alter the normal patterning of the brain. However, the most common form of MR is not linked to metabolic disorders or abnormal brain development, nor any other clinical features and, therefore, is termed non-syndromic MR. An autosomal recessive mode of inheritance may account for nearly a quarter of mentally retarded individuals with non-syndromic MR. However, while 11 genes for

non-syndromic X-linked MR have been found, none of the genes causing non-syndromic autosomal recessive MR had been identified until recently. We analyzed a large consanguineous Algerian family, in which four out of eight children three girls and one boy were affected by a severe impairment of cognitive functions with an IQ below 50 Because the parents were first-degree cousins, we suspected an autosomal-recessive pattern of inheritance. By means of a genome-wide screen with microsatellite markers, we identified a single region of shared homozygosity on chromosome 4q24â€"q25 in the four affected individuals. A detailed sequence analysis of selected genes of this region revealed a 4 bp deletion in the neurotrypsin gene. Neurotrypsin is a neuronal serine protease predominantly expressed in neurons of the cerebral cortex, the hippocampus and the amygdala By immuno-electronmicroscopy, neurotrypsin was localized in the presynaptic membrane and the presynaptic active zone of both asymmetrical excitatory and symmetrical inhibitory synapses Fig. In vitro studies have demonstrated that it is a secreted protein which remains associated with the presynaptic membrane after its secretion. Neurotrypsin shows a unique domain composition It consists of a proline-rich basic segment, one kringle domain, four scavenger receptor cysteine-rich SRCR domains, and a protease domain Fig. The 4 bp deletion, located in the 7th exon, is most likely a null allele as it is predicted to result in a shortened protein lacking the catalytic domain Fig. These findings, therefore, indicate neurotrypsin as the first gene to be identified as a cause of a nonsyndromic autosomal recessive form of MR. A neurotrypsin defect does not appear to be a common cause of MR. We did not find any evidence for neurotrypsin mutations in individuals affected with MR in 17 non-syndromic inbread families. However, we found the same 4 bp deletion in another child born to first-cousin Algerian parents. The two families appear unrelated, but originate from the same area of Eastern Algeria. In both families, the mutation was carried on the background of the same haplotype across the neurotrypsin locus, suggesting a founder effect in the Algerian population. The pathophysiological phenotype and the age of onset of the disease in affected individuals is consistent with the function of neurotrypsin as a regulator of adaptive synaptic functions, such as synapse reorganization during later stages of neurodevelopment and adult synaptic plasticity. In all affected children the course of the disease was similar. They reached the milestones of normal psychomotor development in the first 18 months. Signs of mental retardation were first observed by their parents when they were around 2 years of age. This suggests that neurotrypsin function is crucial in later stages of brain development. Normal cerebral MRI indicates the absence of gross neurodevelopmental abnormalities and a normal ratio between gray and white matter. Taken together, these results provide the first evidence for an association between cognitive impairment and defects in extracellular proteolytic activity at the synapse, opening a novel field in the pathophysiology of mental retardation. The recent generation of mice deficient in the catalytic domain of neurotrypsin in CNS neurons and of mice over-expressing neurotrypsin will provide further insight into the function of this protein. This novel familial disease has first been reported in two Caucasian families living in the USA. In the larger family, affected individuals presented clinically around the fifth decade of life with cognitive decline, including deficits in attention and concentration, difficulties in response regulation, and impaired visuospatial skills. Memory was also impaired, but to a lesser degree than is typically seen in patients with Alzheimer disease. After several years of disease progression, most affected individuals were unable to work and eventually required nursing-home care. The second family showed an earlier clinical onset, during the second or third decade of life. Affected individuals presented with both epilepsy and progressive cognitive decline that eventually required hospitalization. Cognitive changes in mildly to moderately affected subjects were characteristic for deficits in processes dependent on frontal areas. The main neuropathological finding in the two families was the presence of eosinophilic neuronal inclusions distributed throughout the deeper layers of the cerebral cortex and in many subcortical nuclei, especially in the substantia nigra. Biochemical analysis of the inclusion bodies purified from a postmortem brain revealed neuroserpin as the single major protein. Molecular analysis of the neuroserpin gene allowed Davis and colleagues to identify a single point mutation in each family a serto-pro and a serto-arg mutation, respectively Three other families with neuroserpin mutations have been described since. In one of these families with two affected family members, the same serto-arg

mutation was found, although the two families were unrelated. The two other families bear different neuroserpin mutations glyto-glu and histo-arg, respectively Interestingly, all disease-causing mutations known so far are clustered in the so-called shutter region Fig. Neuroserpin is a serine protease inhibitor of the serpin family that has been identified as an axonally secreted glycoprotein in neuronal cultures of chicken dorsal root ganglia In vivo, it is first expressed during late stages of neurogenesis and it has been postulated that neuroserpin plays a role during later stages of synaptogenesis. Persistence of neuroserpin expression during adulthood also suggests a role in adult synaptic plasticity. In vitro studies with cultured hippocampal neurons demonstrated that transcription of neuroserpin mRNA is regulated by electrical activity and increased by depolarization with elevated extracellular KCl. Neuroserpin may act as an activity-regulated modulator of proteolytic activity supporting the hypothesis that proteolytic processes at CNS synapses are involved in mechanisms regulating or realizing structural changes associated with synaptic plasticity In addition, mice overexpressing neuroserpin in CNS neurons under the control of the Thy-1 promoter and mice deficient in neuroserpin have been generated Both deviations from normal neuronal neuroserpin expression result in a marked disturbance of the explorative behavior and severe neophobia The mutated forms of neuroserpin resulting in FENIB deviate from wild-type neuroserpin both in enzymological and physicochemical characteristics. All four mutations found in FENIB are thought to induce neuroserpin polymerization by loopâ€”sheet interaction followed by precipitation. For the serto-pro mutation, a pronounced reduction of the proteinase inhibitor function compared to wild-type neuroserpin has been determined Although for mutated neuroserpin no functional studies are available at the cellular and the systemic level, it is generally accepted that intracellular precipitation and formation of Collins bodies formed by intracellular precipitation of neuroserpin underlies the clinical manifestation. This is best supported by the strong link between the extent of the conformational changes associated with the tendency to precipitate and the onset and the severity of dementia Based on molecular modeling, a functional role of these mutations for the stability of the molecule was predicted. The extent of the conformational changes with regards to the propensity of the mutated protein to aggregate that were predicted for the four mutations showed a clear correlation with the age of onset and the severity of dementia in the affected individuals of the five families. The progressive myoclonus epilepsy that dominates the clinical picture during the early stages of the more severe forms of FENIB is thought to be due to a preferential degeneration of inhibitory neurons. Sonderegger, unpublished data , degeneration due to intracellular neuroserpin precipitation could affect inhibitory neurons prior to excitatory neurons, resulting in a disrupted balance between excitatory and inhibitory activites in the affected regions. A dysfunction of inhibitory neurons could alternatively be explained by a reduced proteinase inhibitory function of mutated neuroserpin However, recently generated mice deficient in neuroserpin exhibit a pronounced difference in their exploratory behavior and an abnormal reaction to novel objects, but no seizures or any other signs of altered excitability In summary, FENIB shows a clear genotype-phenotype correlation with the severity of the disease correlating with the propensity of the mutated neuroserpin to form polymers, demonstrating that intracellular protein aggregation is responsible for neurodegeneration Both overexpression and inactivation of neural protease nexin-1, a serine protease inhibitor of the serpin family, altered hippocampal LTP Finally, abnormalities of both neurons and synapses have been observed in neuropsin-deficient mice These genes are therefore good candidates for being involved in other mental retardation syndromes. Another interesting point is that alterations of the human Reelin gene is associated with an autosomal recessive form of lissencephaly Lissencephaly is a severe developmental disorder in which neuronal migration is impaired, leading to a thickened cerebral cortex whose normally folded contour is simplified and smooth. The Reelin protein is essential for correct cytoarchitectonic organization during CNS development. Mutations of the Reelin gene in mouse disrupts neuronal migration in several brain regions and gives rise to functional deficits, such as ataxic gait and trembling. Thus, reelin is thought to control cellâ€”cell interactions critical for cell positioning in the brain. Its function in adult brain is far less well understood, but altered brain and blood reelin levels have been reported in some psychiatric disorders. An involvement of the reelin signaling pathway in neurodegeneration

has been suggested. Recently, it was reported that reelin has proteolytic activity.

Chapter 6 : Serine Proteases

Serine Proteases and Their Serpin Inhibitors in the Nervous System Regulation in Development and in Degenerative and Malignant Disease Serine proteases in the.

Chapter 7 : Serpin - Wikipedia

It is becoming increasingly clear that the interplay of serine proteases, their serpin inhibitors, serine protease expressed in the nervous system.

Chapter 8 : Proteases & Inhibitors: R&D Systems

Widely recognized as components of the blood coagulation cascade, serine proteases and their natural inhibitors, specific serpins known as the protease nexins, also regulate the maintenance of.