Cystatin C: a potential target for Alzheimer's treatment

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Alzheimer's disease is one of the most prevalent chronic diseases of the aging population. Neuropathologically, the disease is characterized by neurodegeneration and the presence of two pathological features, amyloid plaques and neurofibrillary tangles. Amyloid-β (Aβ) is the major constituent of the amyloid plaques. It is a ubiquitously expressed soluble peptide that can form aggregates, either oligomeric or fibrillar, that are neurotoxic. Extensive research focuses on prevention of Aβ aggregation as a possible therapy for the disease. Recent studies have shown that the endogenous protein cystatin C binds soluble Aβ and inhibits Aβ oligomerization and amyloidogenesis, protecting the brain against amyloid-induced toxicity.

While Aβ is the major amyloid-forming peptide in the brains of Alzheimer's disease patients, the cysteine protease inhibitor, cystatin C colocalizes with Aβ predominantly in amyloid-laden vascular walls [1] and in senile plaque cores of amyloid in brains of patients with amyloidoses (such as Alzheimer's disease, Downs syndrome, cerebral amyloid angiopathy, hereditary cerebral hemorrhage with amyloidosis Dutch type and cerebral infarction [2–6]) as well as in brains of nondemented aged individuals [6]. Cystatin C also colocalizes with Aβ deposits in the brains of aged rhesus and squirrel monkeys [7], dogs [8] and transgenic mice overexpressing the human amyloid precursor protein [6,9]. Aβ is a metabolite of the amyloid precursor protein. Overexpression of mutated forms of the amyloid precursor protein gene that are found in familial Alzheimer's disease in the brains of mice results in amyloid plaque deposition. Some proteins associated with amyloid lesions may have a role in the pathological processes leading to amyloidogenesis and neuronal degeneration, and others may bind secondarily to amyloid deposits. It was demonstrated that cystatin C binds to the Aβ region within full-length amyloid precursor protein and that this association does not affect Aβ generation either *in vitro* [10] or *in vivo* [11]. The association of cystatin C with the amyloid precursor protein was recently confirmed by *in vivo* mapping of protein interactions in intact mouse tissue [12]. Moreover, *in vitro* studies have shown a specific, saturable and high affinity binding between cystatin C and Aβ, suggesting that colocalization of cystatin C with Aβ represents a residual effect of this association [10].

Most importantly, it was demonstrated that cystatin C associates with Aβ inhibits Aβ oligomerization and fibril formation [10,13]. The same role of cystatin C was demonstrated *in vivo* using mice that were genetically engineered to produce human cystatin C as well as abundant amounts of Aβ plaques in their brains. Several lines of transgenic mice expressing human cystatin C under control sequences of the human cystatin C gene [11,14], or specifically in cerebral neurons [15], were crossbred with mice overexpressing human amyloid precursor protein. Cystatin C bound to the soluble, nonpathological form of Aβ in the brains and plasma of these mice and inhibited the aggregation and deposition of Aβ plaques

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in the brain [14,15]. The data obtained show that even subtle modifications in cystatin C expression levels in the CNS, or possibly in the periphery, affect amyloid deposition and modify disease progression.

Based on these data, we hypothesize that cystatin C is a carrier of soluble Aβ in body fluids, such as cerebral spinal fluid and blood, as well as in the neuropil. Systemic or localized increase in cystatin C concentration, relative to that of Aβ, would serve to prevent Aβ aggregation and fibril formation. Endogenous levels of cystatin C do not seem to be sufficient to prevent amyloid deposition in diseased brain. Multiple studies have shown changes in cystatin C serum concentrations associated with a variety of conditions, such as chronic kidney disease, urinary infection, cancer, hypertension, cardiovascular disease, rheumatoid arthritis, glucocorticoid treatment, thyroid dysfunction and aging [16]. Factors that affect cystatin C concentrations in the brain are less well understood. Enhanced cystatin C expression has been observed in response to different types of insults to the CNS, such as ischemia [17]. Altered cystatin C trafficking and a reduction in its secretion is known to be caused by two presenilin-2 mutations (M239I and T122R), linked to familial Alzheimer's disease [18]. Moreover, a decreased cystatin C secretion is associated with a polymorphism found in the cystatin C gene (*CST3*) [19–21]. Genetic studies demonstrated a linkage of the B/B homozygosity in *CST3* with an increased risk of developing late-onset sporadic Alzheimer's disease. (For an updated record of all published Alzheimer's disease-associated studies for *CST3,* see 'Alzgene' on the internet site of the Alzheimer Research Forum [101].) Thus, a decreased cystatin C secretion associated with the *CST3* allele reveals a mechanism for the increased risk of late-onset sporadic Alzheimer's disease conferred by this polymorphism and suggests that a reduced cystatin C brain concentration is associated with the disease [17].

Cystatin C is found in all mammalian body fluids and tissues and has a broad spectrum of biological roles [1]. In the brain, it has been implicated in the response to neuronal degeneration and repair of the nervous system (for review, see [17]). Multiple studies, mainly *in vitro*, propose that cystatin C directly protects neuronal cells from a variety of toxic insults, including against the neurotoxicity induced by oligomeric or fibrillar forms of Aβ. We hypothesize that endogenous cystatin C has an ongoing role inhibiting Aβ oligomerization and amyloidogenesis and protecting against the resultant neurotoxic insults during an individual's lifetime. Studies are underway to identify peptide sequences within cystatin C protein that have anti-Aβ-aggregating activities. These short peptide sequences can serve as templates for drug design of cystatin C peptidomimetic compounds that will have enhanced anti-Aβ aggregation and/or neuroprotective properties.

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Compounds that have the ability to prevent the aggregation of Aβ or that enhance neuronal survival are likely efficacious in inducing disease modification by either delaying the onset of Alzheimer's disease or by arresting and/or slowing disease progression. The use of a peptide analogous to cystatin C sequences that exhibit Aβ carrier characteristics, would serve to develop a novel drug for slowing, halting or reversing disease progression. Furthermore, identification of cystatin C sequences with neuroprotective properties would provide significant beneficial effects for Alzheimer's disease and other neurodegenerative disorders.

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