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QUARTERLY

Review

Targeting BACE with small inhibitory nucleic acids — a future for Alzheimer's disease therapy?★✪

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β-Secretase, a β-site amyloid precursor protein (APP) cleaving enzyme (BACE), participates in the secretion of β-amyloid peptides (Aβ), the major components of the toxic amyloid plaques found in the brains of patients with Alzheimer's disease (AD). According to the amyloid hypothesis, accumulation of Aβ is the primary influence driving AD pathogenesis. Lowering of Aβ secretion can be achieved by decreasing BACE activity rather than by down-regulation of the APP substrate protein. Therefore, β-secretase is a primary target for anti-amyloid therapeutic drug design. Several approaches have been undertaken to find an effective inhibitor of human β-secretase activity, mostly in the field of peptidomimetic, non-cleavable substrate analogues. This review describes strategies targeting BACE mRNA recognition and its down-regulation based on the antisense action of small inhibitory nucleic acids (siNAs). These include antisense oligonucleotides, catalytic nucleic acids — ribozymes and deoxyribozymes — as well as small interfering RNAs (siRNAs). While antisense oligonucleotides were first used to identify an aspartyl protease with β-secretase activity, all the strategies now demonstrate that siNAs are able to inhibit

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Abbreviations: Aβ, β-amyloid peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; CAD cells, catecholaminergic cell line; CNS, central nervous system; CTE, constitutive transport element; miRNA, micro-RNA; RISC, RNA induced silencing complex; RNAi, RNA interference; shRNA, short hairpin RNA; siNAs, small inhibitory nucleic acids; siRNAs, small interfering RNAs.

BACE gene expression in a sequence-specific manner, measured both at the level of its mRNA and at the level of protein. Moreover, knock-down of BACE reduces the intra- and extracellular population of Aβ40 and Aβ42 peptides. An anti-amyloid effect of siNAs is observed in a wide spectrum of cell lines as well as in primary cortical neurons. Thus targeting BACE with small inhibitory nucleic acids may be beneficial for the treatment of Alzheimer's disease and for future drug design.

BACKGROUND

Alzheimer's disease (AD) is the most common cause of dementia in the increasing population of elderly people. According to the World Health Organization about 5.5% of people who live beyond 60 suffer from some form of neurodegeneration. Up to now there are no drugs available that specifically and on a molecular level cure the cause of Alzheimer's disease. Despite many novel therapies investigated in the last decade (donepezil, tacrine, vaccine AN-1792, specific antibodies against AD, and the antibiotic ciloquinol) there is still a need for development of new and more effective treatments of this dementia (Helmut, 2000; Evans, 2001).

Alzheimer's disease is a neurodegenerative disorder characterized by progressive deposition of senile plaques in the brain of patients with ageing dementia. The major component of these aggregates is a 4 kDa β-amyloid peptide (Aβ), a product of proteolytic cleavage of the amyloid precursor protein (APP) (Selkoe,

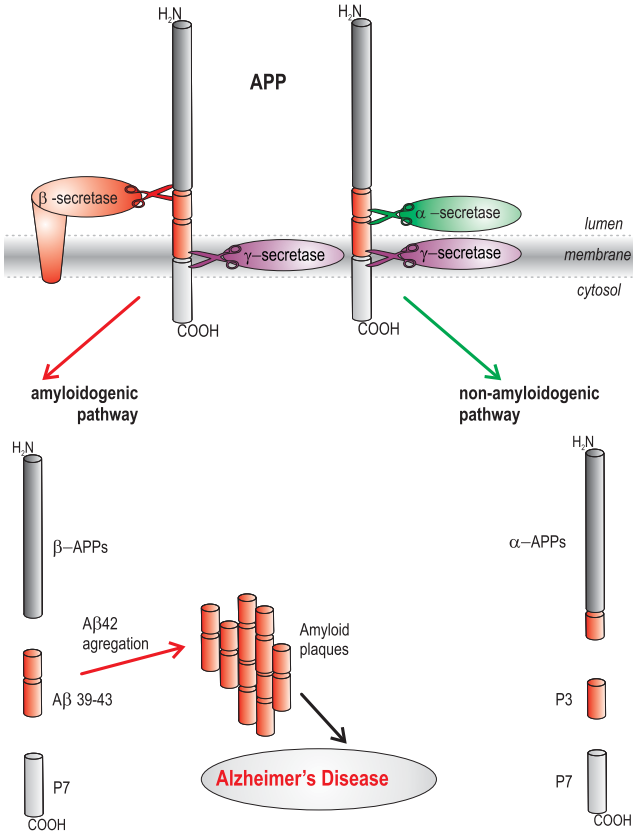

Figure 1. Processing of amyloid precursor protein (APP).

APP proteolysis occurs *in vivo* by two different ways. *Non-amyloidogenic pathway* includes hydrolysis catalysed by α-secretase, resulting in release of soluble α-APPs, and the intra-membrane proteolysis catalysed by γ-secretase secreting peptides P3 and P7. The second, *amyloidogenic pathway* involves β-secretase activity which releases β-APPs, and, after cleveage of the C-terminal fragment by γ-secretase, peptide P7 and Aβ peptides, ranging in length from 39 to 43 amino acids, are secreted. Aβ42 peptide is prone to aggregation and formation of amyloid plaques, accumulation of which is the primary factor driving AD pathogenesis.

1991). The processing of this transmembrane glycoprotein occurs *in vivo* by two different pathways (Fig. 1).

The conventional non-amyloidogenic pathway proceeds *via* proteolytic cleavage of APP by α- and γ-secretases and results in release of non-toxic, soluble α-APPs protein and two other shorter products P3 and P7 (Haass & Selkoe, 1993; Hendriks & Van Broeckhoven, 1996). In normal, healthy individuals these products protect neuronal cells against oxidative stress and participate in wound repair (Mattson *et al.*, 1993; Barger *et al.*, 1995; Mattson & Furukawa, 1997; Kummer *et al.*, 2002). Another APP processing pathway is amyloidogenic. APP is cleaved at the N-terminus of the Aβ region by β-secretase and at the C-terminus by γ-secretase. The cleavage products are β-APPs protein, C-terminal peptide P7 and 39-43 amino acid long beta-amyloid peptide, of which the major products are Aβ40 and Aβ42. The predominant form of Aβ found in the cerebrospinal fluid is the shorter Aβ40. However, the longer Aβ42 peptide, which is released at a much slower rate and thus appears in a much lower concentration, is more prone to aggregation (Jarrett *et al.*, 1993). According to the amyloid hypothesis, accumulation of Aβ is the primary factor driving AD pathogenesis (Hardy & Allsop, 1991; Hardy & Selkoe, 2002). Lowering of Aβ secretion can be achieved by decreasing β-secretase activity rather than by down-regulation of APP substrate protein or γ-secretase inhibition. Therefore, β-secretase is a primary target for anti-amyloid therapeutic drug design (Citron, 2002).

CHARACTERISTICS OF BETA-SECRETASE

The gene encoding β-secretase was sequenced by five independent research groups (Vassar *et al.*, 1999; Hussain *et al.*, 1999; Yan *et al.*, 1999; Sinha *et al.*, 1999; Lin *et al.*, 2000). β-Secretase is an aspartyl protease 2 (Asp2), also called β-site APP cleaving enzyme (BACE) or memapsin 2. This 501-amino acid transmembrane protein contains two active site motifs at amino acids 93-96 and 289-292 (Fig. 2).

These are highly conserved signature sequences of aspartyl proteases, DTGS and DSGT, respectively. The active sites of the protein are situated within the lumenal domain at the right orientation to attack the β-site of APP. The transmembrane domain is located between amino acids 461 and 477. The remaining 24 amino acids of the C-terminal part of the protein are located in a cytosol. BACE contains four N-glycosylation sites and six cysteine residues forming intramolecular disulfide bonds. The optimum activity of BACE is at pH 5.5. β-Secretase catalyses hydrolysis of the peptide bond of APP according to the general mechanism known for aspartyl proteases (Schmidt, 2003). Two aspartic acid residues are involved in catalysis; one of them must be negatively charged. These residues work as acceptors and donors of protons and activate water molecule participating in the peptide bond hydrolysis.

Shortly after BACE was identified, another aspartyl protease, cleaving the APP protein within the β-amyloid sequence, was sequenced (Farzan, 2000). This protein is called BACE2 (memapsin 1, Asp1) and thus BACE was renamed BACE1. BACE1 has 64% sequence identity with BACE2. Both homologues co-express in a variety of cells. BACE1 is present in all tissues with the highest level of expression in the brain (Sinha *et al.*, 1999; Vassar, 1999; Lin *et al.*, 2000). BACE2 is mostly expressed in astrocytes and is not detected in neurons and microglia, where BACE1 is exclusively present (Basi *et al.*, 2003).

BACE1 AS A THERAPEUTIC TARGET

It is a widely accepted opinion that inhibition of Aβ generation by lowering the activity of β-secretase may be beneficial for AD treat-

ment (Citron, 2002a; 2002b). This idea is strongly supported by Roberds *et al.* (2001) who have shown that BACE *knockout* mice are healthy and show no phenotypic differences from their wild-type littermates. Corti-

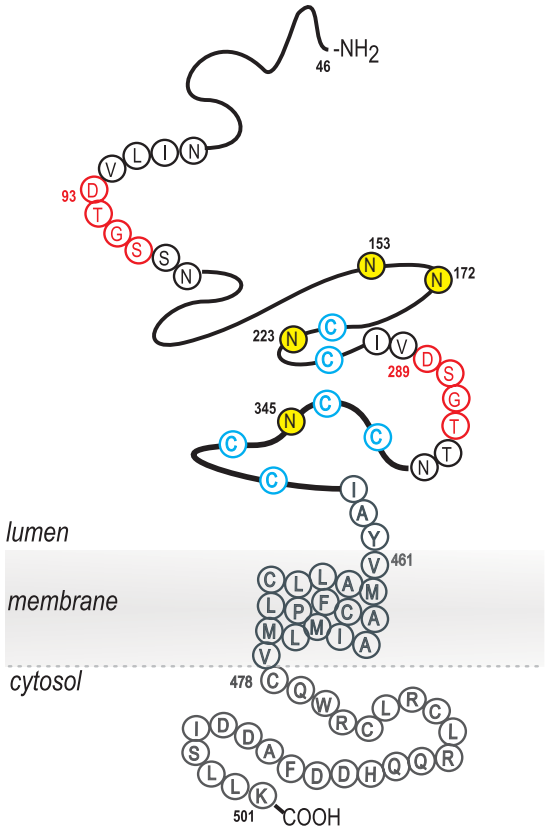

Figure 2. Schematic diagram of aspartyl protease 2 (Asp2, BACE1).

BACE1 is a 501 amino-acid transmembrane protein. Signal peptide (1–21 aa) and pro-peptide (22–45 aa) are cleaved during protein maturation. Within the lumenal domain there are two active site motifs (signature sequences of aspartyl proteases) situated at amino acids 93–96 and 289–292, four N-glycosylation sites and six cysteine residues involved in intramolecular disulfide bonds. The transmembrane domain is located between amino acids 461 and 477, and the C-terminal part of the protein (24 aa) is located in a cytosol.

cal neurons from such mice showed no detectable β-secretase activity and remarkably low levels of Aβ peptide. Moreover, it was shown recently that elevated Aβ production in sporadic Alzheimer's disease patients is correlated with increased β-secretase activity (Li *et al.*, 2004). The level of cholesterol and lipid rafts is probably crucial for activation of β-secretase and β-APP cleavage (Simons & Ehehalt, 2002). Therefore, β-secretase is an excellent target for anti-amyloid therapeutic drug design (Citron, 2002a; 2002b). Several approaches were immediately undertaken in order to find an effective inhibitor of human β-secretase activity (Ghosh *et al.*, 2000; 2001; Turner *et al.*, 2001; Brady *et al.*, 2004). The main strategy for designing BACE inhibitors utilized peptide-based analogues of the substrate. However, due to the 30% sequence identity of BACE1 with other aspartyl proteases the selectivity of BACE peptidomimetic inhibitors is not satisfactory (Ghosh *et al.*, 2000). Several non-peptidomimetic inhibitors have been found which effectively block BACE activity (Miyamoto *et al.*, 2001; Qiao & Etcheberrigaray, 2002; Bhisetti *et al.*, 2002). Two crystal structures of BACE1 complexed with its inhibitors OM99-2 and OM00-3 were solved by Hong *et al.* (2000; 2002). The structure of an active site and APP binding domain is helpful in designing low molecular mass inhibitors for BACE, especially because it revealed that the active site of BACE1 is more open and less hydrophobic than typical sites of other aspartyl proteases. The progress in the search of selective β-secretase inhibitors has been recently summarized in several review papers (Ghosh *et al.*, 2002; Roggo, 2002; Schmidt, 2003; John *et al.*, 2003).

STRATEGIES BASED ON DOWN-REGULATION OF GENE EXPRESSION BY SMALL INHIBITORY NUCLEIC ACIDS

Strategies targeting mRNA recognition and its down-regulation are based on the antisense action of small inhibitory nucleic acids (siNAs) including antisense oligonucleotides, catalytic nucleic acids – ribozymes and deoxyribozymes as well as small interfering RNAs (siRNAs) (Fig. 3). All of these nucleic acids recognize the target molecule *via* sequence-specific Watson–Crick base- pairing

and lead to the formation of a complementary siNA/mRNA duplex. The mechanism of action of particular siNAs is different. Antisense oligonucleotides, when bound to the target molecule form DNA/mRNA duplexes block the translation by "hybridization arrest" or activate RNase H and lead to RNA degradation (Stein & Cheng, 1993). Ribozymes and deoxyribozymes facilitate the cleavage of RNA phosphodiester bonds *via* a catalytic mechanism (Emilsson *et al.*, 2003). Up to now, it has been demonstrated that plasmid-coded ribozymes, coupled on their 5′-ends with a $tRNA^{Val}$ sequence, express intracellularly with high efficiency (Koseki *et al.*, 1999). Such ribozymes, when mimicking the 3′-immature tRNA molecule, are recognized by the nuclear protein *exportin-t* (Xpo-t) and effectively exported from the nucleus to the cytoplasm (Kuwabara *et al.*, 2001). Conjugation of the 3′-end of the ribozymes with an aptameric sequence for cellular helicases, unwinding the intramolecular helices of the messenger RNA, facilitates the high efficiency of ribozymes in *in vivo* experiments, independently of the secondary structure of the target mRNA (Warashina *et al.*, 2001; Kawasaki *et al.*, 2004).

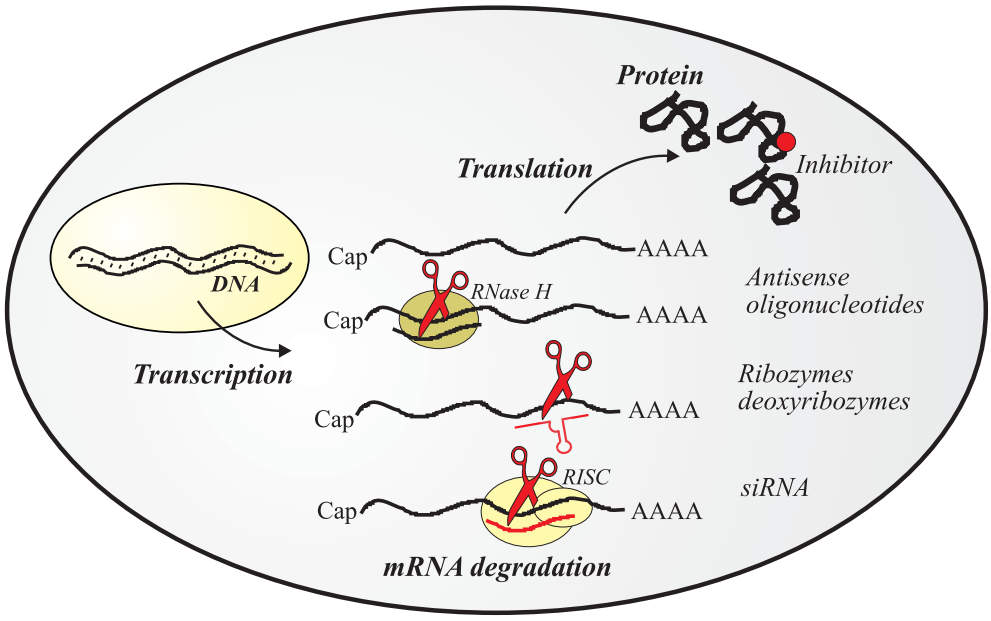

Figure 3. Strategies for down-regulation of gene expression by small inhibitory nucleic acids (antisense oligonucleotides, catalytic nucleic acids and siRNA).

All presented strategies concern mRNA degradation on post-transcriptional level, based on a sequence-specific target molecule recognition. Antisense strategy involves RNase H activation by DNA/RNA hybrid and cleavage of RNA within the sequence of the duplex. Ribozymes and deoxyribozymes exhibit catalytic activity for RNA hydrolysis. Short interfering RNAs (siRNAs) induce gene silencing *via* activation of RISC – a protein complex with helicase/nuclease activity.

Antisense, ribozyme and deoxyribozyme strategies are widely used to design nucleic acids for therapeutic applications (Christoffersen & Marr, 1995; Lewin & Hauswirth, 2001; Opalinska & Gewirtz, 2003). In recent years various antisense oligonucleotides and ribozymes have been the subjects of many pre-clinical and clinical trials (Kurreck, 2003), including *ex vivo* treatment of HIV-1 infected patients (Sullenger & Gilboa, 2002). Moreover, the first antisense oligonucleotide *Vomivirsen* was successfully introduced for the treatment of AIDS patients infected with cytomegalovirus (Highleyman, 1998). Nucleic acid technology has been also considered as a possible therapeutic approach for treatment

of disorders of the central nervous system (CNS). The latest achievements in identifying target genes and the use of inhibition approaches have been summarised recently by Trülzsch & Wood (2004).

Tuschl's discovery in 2001 that chemically synthesized short RNA duplexes (small interfering RNAs, siRNAs) exhibit a potency for sequence-specific cognate gene silencing in mammalian cells *via* an RNAi pathway (Elbashir *et al.*, 2001a) opened a Pandora's box. The search for siRNA sequences able to specifically and efficiently down-regulate gene expression is of interest from several points of view. Besides their relevance for genotype/phenotype relationship investigations siRNAs have been immediately considered as molecular tools for target identification and implemented for therapeutic application (Dorsett & Tuschl, 2004). The mechanism of RNA interference involves activation of the nuclease/helicase protein complex RISC (*RNA induced silencing complex*) and guiding it to the complementary mRNA *via* an antisense strand of siRNA. The nucleolytic activity of the RISC complex leads to cleavage of the target mRNA and, in consequence to the inhibition of protein biosynthesis. The effect of gene silencing can last for several days if siRNA molecules are generated endogenously from plasmids coding siRNAs or short hairpin RNAs (Miyagishi & Taira, 2002; Paddison *et al.*, 2002; Paul *et al.*, 2002; Brummelkamp *et al.*, 2002; Paddison *et al.*, 2002), the substrates of the ribonuclease Dicer (Bernstein *et al.*, 2001). Interestingly, in mammalian primary neurons the effect of gene silencing induced by small interfering RNAs can last for at least three weeks (Omi *et al.*, 2004). It has been postulated that siRNA duplexes that are not fully complementary to the cognate mRNA can induce repression of protein translation using the same RISC complex and micro-RNA mechanism (Schwarz *et al.*, 2003).

Recently, severe non-specific effects have been observed in the profile of gene expression when siRNAs were used at high concentrations (>100 nM) (Bridge *et al.*, 2003; Jackson *et al.*, 2003; Persengiev *et al.*, 2004). However, these off-target effects can be excluded by selection of the best target sites and the lowering the amount of siRNAs applied.

Accessibility of the target mRNA sequences to siNA molecules is one of the limiting factors for efficient down-regulation of gene expression. Even though RNA helicases participate in the siRNA-induced gene silencing, none of small inhibitory nucleic acids have internal potency for unwinding mRNA intramolecular helices, which would facilitate accessibility to complementary target sequences. Some effort has been done to design helicase-attached hybrid ribozymes which are active in cellular systems independently of the secondary structure of the target mRNA (Warashina *et al.*, 2001; Kawasaki *et al.*, 2004). Besides, screening techniques are used to find active antisense, ribozyme and siRNA sequences that are able to suppress, to a reasonable extent, the expression of the cognate gene.

DOWN-REGULATION OF BACE GENE EXPRESSION

Approaches used to inhibit BACE gene expression include applications of antisense oligonucleotides, catalytic nucleic acids and small interfering RNAs. Although each of these strategies works *via* antisense recognition of the target RNA and uses different mechanisms of action, all of them are designed to down-regulate gene expression at the post-transcriptional stage. The secondary structure of the target BACE mRNA molecule (accession number AF190725, start codon 5′-AUG^{456} –3′) generated with the help of the MFOLD program (Zuker, 2003) is shown at Fig. 4.

An antisense approach was used by Yan *et al.* (1999) to identify an aspartyl protease with the β-secretase activity. Experiments

were done in HEK293 cells overexpressing APP695 protein with the Swedish mutation KM → NL, (which makes the substrate protein more prone to β-cleavage), and with two lysines at the C terminus (HEK293sw-KK cells). Two of the antisense oligomers directed toward aspartyl protease mRNA significantly reduced the BACE message level (Table 1). The amounts of Aβ peptides 1–40 and 1–42 released into the culture medium were also reduced by 50–80%. Similar BACE gene down-regulation and lowering of Aβ production were observed in human neuroblastoma IMR-32 and murine Neuro-2A cells. These experiments proved that aspartyl protease BACE is involved in Aβ secretion in both somatic and neuronal cells.

Similarly, Vassar *et al.* (1999) used a set of antisense oligonucleotides to establish the necessity of BACE for the APPsw cleavage at the β-site. The 25 nt long antisense oligonucleotides (entry 7–9, Table 1) reduced the BACE mRNA level by up to about 25–30%; while extracellular Aβ40 and Aβ42 peptides were secreted in about 60–70% yield in comparison to the control oligomer-treated cells.

An approach based on RNA-cleaving ribozymes was developed to control expression of β-secretase (Nawrot *et al.*, 2002; 2003). Two sites of BACE mRNA were chosen as tar-

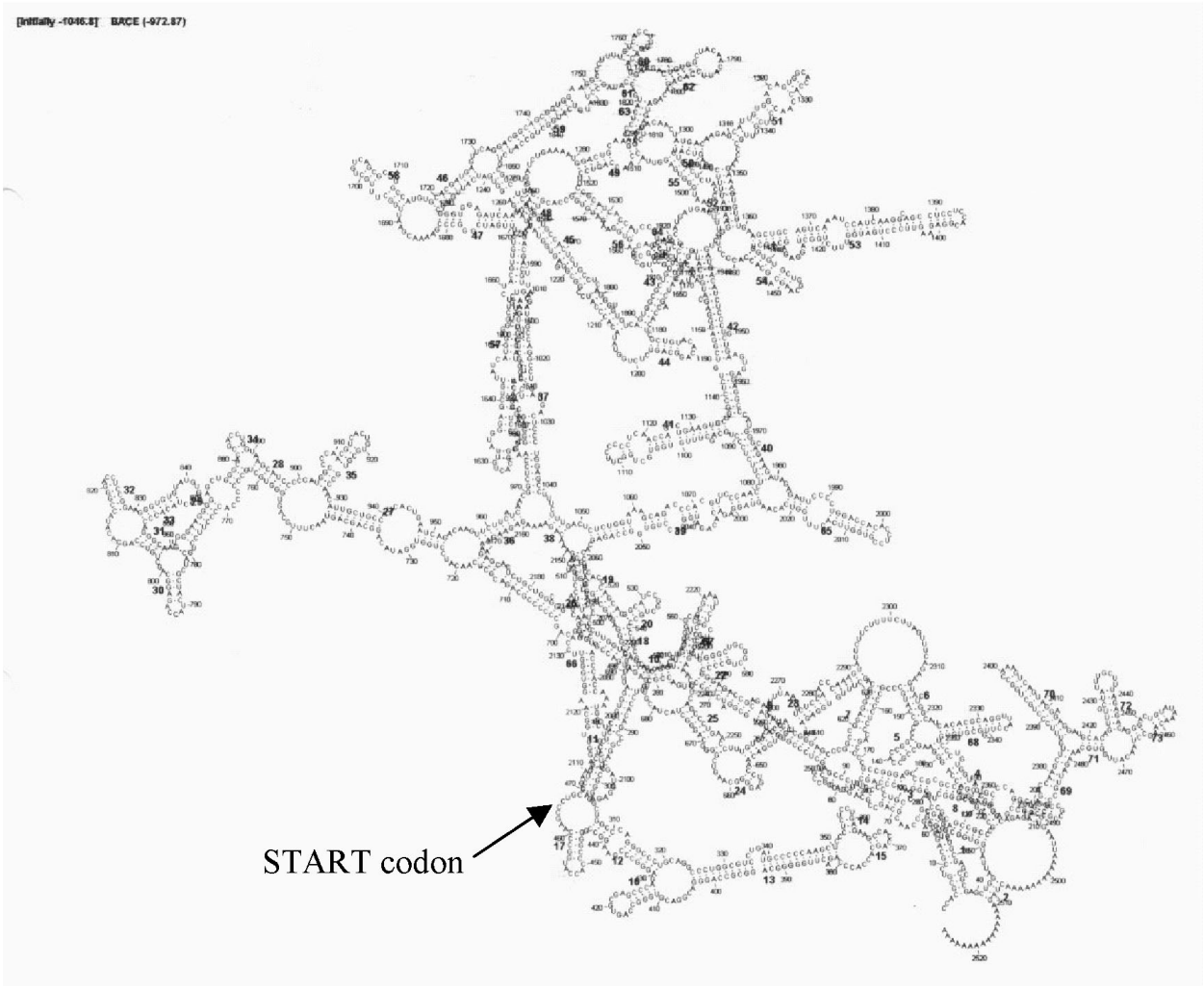

Figure 4. Secondary structure of β-site APP cleaving enzyme (BACE1) mRNA (accession number AF190725, start codon 5′-AUG^{456}–3') generated with the MFOLD program (Zuker, 2003).

get sequences for endogenously generated ribozymes. The ribozyme cassette was designed to constitute a catalytic *hammerhead* core and substrate recognition arms, flanked at the 5′-terminus with $tRNA^{Val}$ and at the 3′-terminus with CTE (*constitutive transport element*) sequences. Ribozyme cassettes were cloned into a pUC19 plasmid and used for transient transfection of HEK293 cells. Such ribozymes efficiently inhibit BACE gene expression at the level of mRNA (up to 95%) and at the level of protein (up to 90%). Inhibition of BACE activity directly influences the intra- and extracellular population of Aβ40 peptide (Table 1, entries 10 and 11). A similar anti-amyloid effect was observed in experiments of BACE down-regulation performed on a SH-SY5Y human neuroblastoma cell line (Nawrot *et al.,* unpublished). Therefore, such ribozymes, especially when coded in viral vectors, may be considered as inhibitors of β-secretase activity, and further, as diagnostic and therapeutic agents for AD treatment.

Table 1. Approaches for inhibition of BACE gene expression in cultured cell lines (BACE mRNA – accession number AF190725).

siNA	Cell line	Entry	Range of the target sequence 5' → 3'	% of Inhibition mRNA	% of Inhibition Aβ40 (Aβ42)	Reference
chimeric	HEK293/	1	1613—1637	>80	72(68)	
antisense	APPsw-KK	2	1707—1731	>80	68(52)	Yan *et al.*,
chimeric	IMR-32	3	1613—1637	75	51(58)	1999
antisense		4	1707—1731	39	57(56)	
chimeric	Neuro-2A	5	1613—1637	>60	70(67)	
antisense	/APPsw-KK	6	1707—1731	>60	61(61)	
antisense	101 / APPsw	7	1702—1736	74	37(27)	Vassar *et al.*,
		8	2017—2041	74	37(37)	1999
		9	2174—2198	70	27(21)	
	HEK293	10	655—675	>90	>80	Nawrot *et al.*,
hammerhead		11	815—835	>90	>80	2002, 2003
ribozyme	SH-SY5Y	12	655—675	90	90	Nawrot *et al.*, (unpublished)
DNAzyme 10-23	HEK293	13	2295-2315	~70		Nawrot *et al.*, (unpublished)
	A549	14	1499—1517	90		McSwinggen &
siRNA		15	1762—1780	40		Beigelman,
(3'-TT)		16	2466—2484	90		2003
		17	3585—3603[a]	70		
	HEK293 /	18	709-728	~60		
siRNA	APPsw					Basi *et al.*,
(3'-TT)	HEK293 /	19	1267-1286	~50[b]		2003
	APPwt /	20	1658-1677	~50[b]		
	BACE1	21	709-728	>90[b]	~50	
siRNA (3'-TT,	CAD/	22	629-648	99[b]		
5'-phosphate)	APPwt/	23	711-730	~70[b]		
or siRNA in	BACE1	24	1280-1299	90[b]		
pSilencer vector		25	1691-1710	~50[b]		
siRNA (3'-TT,	mPCNwt[c]	26	629-648	70[b]	59(57)	Kao *et al.*,
5'-phosphate)		27	711-730	70[b]	61(49)	2004
		28	1280-1299	70[b]	50(46)	
		29	1691-1710	70[b]	43(40)	
	mPCN-	30	629-648	>90[b]	41(42)	
	APPsw[d]	31	1280-1299	>90[b]	36(42)	

[a]BACE accession number NM_012104; [b]as the amount of recombinant BACE protein; [c]primary cortical neurons of wild type mice; [d]primary cortical neurons of the APPsw transgenic mice.

Several DNAzymes (deoxyribozymes) 10–23, complementary to BACE mRNA, were screened for their cleavage efficiency. A few of the molecules were active in the cellular test system (HEK293 cells), reducing BACE

gene expression by 50–70% (Nawrot *et al.,* unpublished). Further experiments are necessary to demonstrate the influence of the active deoxyribozymes at the level of the BACE protein and the released β-amyloid peptides.

Small nucleic acid molecules, such as short interfering nucleic acids (called also siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA) and short hairpin RNA (shRNA), capable of mediating RNA interference against BACE gene expression, were patented by McSwinggen & Beigelman (2003) as an approach to the treatment of Alzheimer's disease. The patent also covers chemically modified siRNAs, comprising e.g. 2′-deoxy-2′-fluoro or 2′-amino pyrimidine and purine nucleotides, in which the sense strand was additionally modified with 5′- and 3′-terminal inverted deoxyabasic caps and the antisense strand comprised a 3′-phosphorothioate internucleotide linkage. Four siRNAs directed toward BACE mRNA sequences are illustrated (entry 14–17, Table 1). The siRNAs, containing 19 nt non-modified RNA duplexes 3′-terminated with double thymidine overhangs, were used for lipid-assisted transfection of human lung cancer A549 cells. The siRNAs, at a final concentration of 25 nM, proved to be very efficient down-regulation agents, reducing the level of BACE messenger RNA by up to 90% of that in control cells transfected only with lipofectamine.

Small interfering RNAs directed toward endogenous and plasmid expressed BACE1 and BACE2 genes were used by Basi *et al.* (2003) to investigate the role of endogenous BACE2 protein in the production of Aβ peptide. To induce BACE1 gene silencing by RNA interference, three synthetic siRNAs, designed as recommended (Elbashir *et al.,* 2001b), were tested in HEK293 cells stably transfected with wild type APP or APPsw genes. The siRNA, directed toward sequence 709–728 nt of BACE1 mRNA, suppressed endogenous message level up to 36%, and specifically reduced the amount of overexpressed BACE1 protein (>90%). siRNA targeting BACE1 led to a decrease in secreted sAPPβ as well as Aβ peptide, consistent with earlier cellular and *in vivo* studies (Roberds *et al.,* 2001; Cai *et al.,* 2001; Luo *et al.,* 2001). Moreover, siRNA to BACE1 did not affect expression of BACE2 and *vice versa*. Silenced BACE1 and non-altered BACE2 gene expression was beneficial for the reduction of Aβ, proving the antagonistic activities of both proteases (Basi *et al.,* 2003).

Although there is 64% homology between the aspartyl proteases BACE1 and BACE2, Kao *et al.* (2004) designed four siRNA sequences targeted to BACE1 which specifically and selectively suppressed BACE1 and not BACE2 expression in various cell systems (entry 22–25, Table 1). First, the activity of the siRNAs was tested in a catecholaminergic cell line (CAD cells) overexpressing APP and BACE1 proteins. Two of the selected siRNAs demonstrated >90% efficacy on suppressing recombinant BACE1, and the others (entry 23 and 25) down-regulated the gene of BACE by 70 and 50%, respectively. Similar effects of BACE1 silencing were observed in this system when siRNAs were generated from short hairpin RNAs coded in a pSilencer vector. Secondly, it was also demonstrated that siRNAs directed toward sequences 22 and 24 (Table 1) significantly reduced the level of endogenously expressed BACE1 in HEK293 cells (Kao *et al.,* 2004). Furthermore, selected siRNA molecules reduced Aβ production in primary cortical neurons derived from both wild type and APPsw transgenic mice. While siRNAs used in all test systems significantly reduced the secretion of Aβ40 and Aβ42, there were no changes in APP and presenilin 1 (PS1) protein levels and their subcellular distribution. Importantly, pre-treating neurons with BACE1 siRNAs reduced H_2O_2 induced cell death, and thus the neurotoxicity of Aβ caused by oxidative stress could be significantly decreased.

BACE KNOCKOUT: HOPES AND HYPES

As has been shown in cellular systems and in *in vivo* experiments, BACE1 mRNA knockout is directly linked with protein expression, as well as with APP metabolite production. The down-regulation of BACE1 by small inhibitory nucleic acids specifically impacts on the β-cleavage of APP and Aβ production in both, somatic and neuronal cells. Although the use of siNA may constitute a potential anti-amyloidal approach to the treatment of Alzheimer's disease there are several disadvantages for BACE1 targeted therapies. β-Secretase, like the other transmembrane proteases, may have multiple substrates. Two of them, an α-2,6-sialyltransferase (ST6Gal I) and P-selectin glycoprotein ligand-1 (PSGL-1) have been identified by Kitazume *et al.* (2001) and Lichtenthaler *et al.* (2003), respectively. These findings indicate that only partial BACE1 down-regulation would be beneficial to other BACE1 substrates. BACE1 exhibits >30% sequence homology with other aspartyl proteases of the pepsin family, and about 60% sequence identity with its BACE2 homologue. BACE1 inhibitors cross-interacting with other aspartyl proteases have to be excluded as AD therapeutics. Besides specificity, the delivery of small inhibitory nucleic acids to tissues and primary neurons of the central nervous system (CNS) hampers progress in the design of novel oligonucleotide therapeutics.

However, it has been demonstrated that intact antisense oligonucleotides crossed the blood-brain barrier (BBB) in mice overexpressing APP (Banks *et al.*, 2001). The recent report that oligonucleotides encapsulated with nanogels (polyethylene glycol and polyethyleneimine) can effectively cross the BBB both in cellular and in mouse models (Vinogradov *et al.*, 2004) kindled some hopes that this obstacle can be overcome. Also the recent reports that virus-based vectors are effective carriers of gene/siNA therapy directed toward the CNS in *in vivo* experiments (Pardridge, 2002; Davidson & Breakefield, 2003; Trülzsch & Wood, 2004) keep that therapeutic strategy still promising.

In summary, the sequence-specific down-regulation of BACE1 results in lowering of b-secretase activity and decreasing of Ab formation. Thus, small inhibitory nucleic acids, including antisense oligonucleotides, catalytic nucleic acids and small interfering RNAs can be considered as future anti-amyloid agents useful in designing novel Alzheimer's disease therapies.

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