SUPPLEMENTAL DATA

Antisense Tools for Functional Studies of Human Argonaute Proteins

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Table S1: AsONs designed and tested in this study

Target	Name[a]	Sequence of the asON (5´ to 3´)	Efficacy[b]	Selectivity[c]	IC_{50} (nM)
hAgo1	as343/21 = asON1	GATCTTAGGGATGTCCACCTC	+	+	~ 9
	as365/20	CACCTCGTAGTGGTACACGT	+	+	~ 13
	as933/20	GCTTGGGCTGCTCATCTATG	+	-	~ 21
	as2339/22	TAGCTCATAGTGGAGTATCTGG	-	n.d.	n.d.
hAgo2	as140/20	AATTTGATTGTTCTCCCGGA	-	n.d.	n.d.
	as1295/19	TATTCCTGCCCCCGTAGAG	+	-	~ 70
	as2269/20	GGTAGAAGTCGAACTCGGTG	-	n.d.	n.d.
	as2953/20	TTAAAAACAAGTAAATTGAA	-	n.d.	n.d.
	as2966/20	GAGAATCATGTATTAAAAAC	-	n.d.	n.d.
	as2978/20	ATCAATTTCATAGAGAATCA	-	n.d.	n.d.
	as3022/24	GTTTTGTGTTGCTTTCACTCTCAG	+	n.d.	n.d.
	as3024/24 = asON2	GTGTTTTGTGTTGCTTTCACTCTC	+	+	~ 7
	as3024/22	GTTTTGTGTTGCTTTCACTCTC	+	n.d.	n.d.
	as3025/21	GTTTTGTGTTGCTTTCACTCT	+	n.d.	n.d.
	as3340/20	GATGATTAGACTGTCAGTAT	-	n.d.	n.d.
	as3327/25	GACTGTCAGTATATTGTCTTTTTTC	-	n.d.	n.d.
hAgo3	as787/20 = asON3	AGAAAAATGAACGCCCCACA	+	+	~ 11
	as799/20	CTTCTGGAGCGGAGAAAAAT	+	n.d.	n.d.
	as1264/20	CTAGTGGCAGGTAGGTGTGT	+	n.d.	n.d.
	as1271/20	CAGACTTCTAGTGGCAGGTA	-	n.d.	n.d.
	as1284/23	CCCTGCCACAATATTACAGACTT	-	n.d.	n.d.
	as1291/20	GTTGCCCTGCCACAATATTA	+	+	~ 10
	as2642/20	GCAGGTATAGAAACAGATCG	-	n.d.	n.d.
	as2644/20	GTGCAGGTATAGAAACAGAT	-	n.d.	n.d.
	as2650/20	CGCTGGTGCAGGTATAGAAA	-	n.d.	n.d.
hAgo4	as1842/20 = asON4	CAGGCAAAGATTGGAAAGGG	+	+	~ 24
	as1861/20	CAAGTTTTGCATTTATCTTC	+	n.d.	n.d.
	as3996/20	TGCAAGTAGTCGTGTGAGTT	+	n.d.	n.d.
	as4005/20	AATTTGTACTGCAAGTAGTC	+	n.d.	n.d.
	as4510/20	GTTTATGAGATTTGGACCGT	+	+	~ 15
	as4520/20	GAGAAGGCTAGTTTATGAGA	+	n.d.	n.d.

[a]Numbers indicate the position on the target mRNA of the first matching nucleotide from the 5´end with the asON and the length of the asON.
[b]The efficacy of asON was determined via RT-qPCR, 24h after transfection with 100nM asON of ECV304 cells. Levels of target mRNA expression normalized to mRNA amount in asON_Ctrl treated cells (set 100%) ≤ 40% (+) or > 40% (-) are indicated.
[c] High selectivity (+) or unspecific downregulation of other hAgo mRNA (-) are indicated.
n.d.: not determined.

Table S2: Binding sites for the asONs on hAgo transcripts

Target	asON	Target accessible site[a] (5´ to 3´)
hAgo1	asON1	UUACUUUGAGGUGGACAUCCCUAAGAUCGACGUG
hAgo2	asON2	TCTTCTGAGAGTGAAAGCAACACAAAACACAAGTGT
hAgo3	asON3	GAAATACACACCTGTGGGGCGTTCATTTTTCTCCGC
hAgo4	asON4	ACCCTTTCCAATCTTTGCCTGAAGATAAATGCAAAAC

[a] The four asONs were designed to target accessible sites (underlined nucleotides) on the hAgo transcripts and their matching sequences are labelled in red.

Supplemental Figure Legend

FIGURE S1. Cytotoxicity of the asONs developed in this study. ECV304 cells were transfected with 0-1,000 nM of each asON, then 24h (*A*) or 48h (*B*) after transfection a CytoTox-Glo™ Cytoxicity Assay (Promega), which measures a distinct protease activity released by dead cells, was performed following manufacturer instruction. Briefly, cells were transfected using 5 µg/ml Lipofectamine2000 (Invitrogen) in 96-well plates (15,000 cells/well) with asON (40µl final volume of the transfection solution). After 24h or 48h dead-cell protease activity (corresponding to the amount of cell death) was measured in the culture medium. Then cells were lysed, total protease activity was measured (corresponding to the total amount of cells/well), and the percentage of dead cells was then calculated. Indicated are mean values and standard deviations.

FIGURE S2. SiAgos downregulate efficiently their target mRNA. ECV304 cells were transfected with 100 nM siAgo, then 24h after transfection cells were collected, the total RNA was isolated and reverse transcribed. The cDNA was quantified by RT-qPCR and a house-keeping gene, beta glucoronidase, was used as internal control. As control siscr was used which has no endogenous target. Indicated are mean values and standard deviations.

FIGURE S3. Downregulation of hAgo1 and hAgo2 at the protein level by asON1 and asON2, respectively. ECV304 cells were transfected with 100 nM asON1 (left panel) or 100 nM asON2 (right panel). Cells were collected at different time points, lysed and a Western blot analysis was performed using rat-anti human Ago1 and Ago2 antibodies. Values were normalized to beta actin, which was used as loading control, and to hAgo expression levels in asON_Ctrl treated cells which were set 100.

FIGURE S4. The asONs and siAgos show a high efficiency in downregulating their target mRNA also when used in combinations. ECV304 cells were transfected with 100 nM of each asON (*A*) or siAgo (*B*), then 24h after transfection cells were collected, the total RNA was isolated and reverse transcribed. The cDNA was quantified by RT-qPCR and a house-keeping gene, beta glucoronidase, was used as internal control. As control for the asON, asON_Ctr was used which has a fully-modified phosphorothioate backbone, while as control for the siAgos, siscr was used. Both controls have no endogenous target and were used in an amount reflecting the total quantity of transfected nucleic acid. Indicated are mean values and standard deviations.

FIGURE S5. Downregulation of hAgo1 (*A*) and hAgo2 (*B*) at the protein level is efficient also when asONs or siAgos are used in combinations. Downregulated hAgos are indicated with (+), while the not suppressed species are indicated with (-). ECV304 cells were transfected with 100 nM each asONs (upper panel) or 100 nM each siAgos (lower panel). Cells were collected after 48h, lysed and a Western blot analysis was performed using rat-anti human Ago1 and Ago2 antibodies. Beta actin was used as loading control. In these experiments, a new batch of antibody directed against hAgo2 was used which led to detection of three bands corresponding to hAgo2. Therefore, hAgo2 is here indicated with a bar.