Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 361 (2007) 14–19
BBRC
www.elsevier.com/locate/ybbrc

Platination of the siRNA sense-strand modulates both efficacy and selectivity *in vitro*

Margareta Hägerlöf, Hanna Hedman, Sofi K.C. Elmroth *

Biokemi, Kemicentrum, Lunds Universitet, Box 124, SE-221 00 Lund, Sweden

Received 7 June 2007
Available online 6 July 2007

Abstract

The use of short interfering RNAs (siRNA) for selective suppression of protein production has rapidly become a commonly used technique for transient modulation of protein levels. In the present paper, we investigate whether introduction of platinated bases in the sense strand can be used to modulate the efficacy of siRNAs. Four different siRNAs were studied, all targeting the initial AU-rich 3′ UTR of Wnt-5a mRNA. The siRNAs were characterized with respect to melting properties and translational inhibitory effect *in vitro* using luciferase as a reporter gene. The translation inhibition studies reveal that all platinated siRNA remain efficient. For an siRNA with partial complementarity to the luciferase gene, platination was shown to reduce the off-target effects. All siRNAs were found to be active in cellular *in vitro* translation systems, reaching suppression levels well above 80% for the majority of siRNAs investigated.

Keywords: siRNA; Off-target minimization; Platinum; Wnt-5a; 3′ UTR; RNA; Down regulation

The use of short interfering RNAs (siRNAs) for transient suppression of protein production has become a commonly used molecular biology technique holding promise also for therapeutic use [1,2]. The technique relies on use of the endogenous RNA induced silencing complex (RISC) for processing and transport of the antisense strand of the siRNA to its complementary mRNA target [3,4]. For optimal processing, several factors have to be considered during siRNA design, the more important ones being (i) mRNA target accessibility and (ii) use of siRNA duplexes with melting properties favouring loading of the antisense strand to RISC. In the latter case, siRNAs with hybridization properties favouring local melting of the 5′ end of the antisense strand over melting of the 3′ end have been shown to be particularly efficient [5–9]. The observation fits well with a picture of the process where the 5′ ends of the siRNAs compete for loading onto RISC. For highly symmetrical siRNAs, loading of both sense- and antisense-strands might thus occur, with significant off-target effects as the result of the unwanted sense–mRNA interaction.

For use of siRNAs as a transient gene knock-down reagent, the requirement of a low-melting antisense 5′ end can usually be fulfilled, since the target mRNA can be chosen to fit this criteria. However, when the mRNA region of interest is limited, or the siRNA is used to interfere with for example AU-rich regions in the untranslated regions (UTR) of the mRNA, the requirement of a low-melting 5′ end might be more difficult to fulfil. In some cases, introduction of mismatches have been used to modulate the melting properties of otherwise unfavourable siRNAs to facilitate loading of the antisense strand to RISC [10–13]. However, since the use of mismatches for targeting of AU-rich sequences is likely to increase also the probability of microRNA-like (miRNA-like) induced activity [14,15], i.e., translational block rather than mRNA degradation, an alternative approach is highly warranted.

The aim of the present study has been to evaluate whether pre-platination of the sense strand of siRNAs can be used as a method to modulate siRNA efficacy. For this purpose, the initial AU-rich 3′ UTR of Wnt-5a

* Corresponding author. Fax: +46 46 222 4116.
E-mail address: sofi.elmroth@biochemistry.lu.se (S.K.C. Elmroth).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.06.131

mRNA was chosen as a target for the siRNAs employed [16,17]. The region belongs to a part of the Wnt-5a mRNA which serves as a potential protein binding site for HuR, a process that has been suggested to prevent the mRNA from degradation [18–20]. The four siRNAs studied here have recently been shown to reduce protein production in both cell free and cellular *in vitro* test systems when used as unmodified double-stranded siRNA [21]. Our present data reveals that platination of the sense-strand is compatible with siRNA activity with either similar or improved activity compared to the unmodified siRNAs.

Materials and methods

Chemicals and buffers. Autoclaved and sterile filtered H_2O of Millipore quality was employed for all applications. Cisplatin (*cis*-$Pt(NH_3)_2Cl_2$) was bought from Sigma. A MOPS buffer (50 mM MOPS, 140 mM NaOAc, 2 mM $Mg(OAc)_2$, pH 6.3) or phosphate buffer (100 mM NaCl (Sigma), 20 mM Na_2HPO_4/NaH_2PO_4 (Sigma) pH 6.5) was used for pH control. Cells were cultured in Dulbecco's Modified Eagle's medium (DMEM, Sigma) supplemented with 10% fetal bovine serum, 10 μg/ml bovine insulin, 5 μg/ml hydrocortisone, 5 units/ml penicillin, 0.5 units/ml streptomycin, and 2 mM L-glutamine (Normal DMEM). Transfection was performed in Lipofectamine 2000 (Invitrogen) in Dulbecco's modified Eagle's medium supplemented with 10 μg/ml bovine insulin, 5 μg/ml hydrocortisone, and 2 mM L-glutamine (Transfection DMEM), i.e., DMEM lacking serum and antibiotics.

Oligonucleotides. All oligonucleotides were purchased from IBA (Germany) and PAGE purified by the manufacturer. The siRNAs were designed as 19 nucleotide RNA duplexes with a d(TT) 3′ overhang and an unmodified 5′-hydroxyl end. The siRNAs were numbered with respect to the location of their upstream, starting 5′ hybridization site within the 3′ UTR of Wnt-5a mRNA (GenBank accession no. NM_003392), the latter renumbered from one starting at the base directly following the stop codon (base 1462). The sequences used were the following: W-siRNA33 (sense: 5′-GGA CCC GCU UAU UUA UAG ATT-3′, antisense: 5′-UCU AUA AAU AAG CGG GUC CTT-3′), W-siRNA139 (sense: 5′-CCA UCU AAG AAC UCU GUG GTT-3′, antisense: 5′-CCA CAG AGU UCU UAG AUG GTT-3′), W-siRNA147 (sense: 5′-GAA CUC UGU GGU UUA UUA UTT-3′, antisense: 5′-AUA AUA AAC CAC AGA GUU CTT-3′), W-siRNA156 (sense: 5′-GGU UUA UUA UUA AUA UUA UTT-3′, antisense: 5′-AUA AUA UUA AUA AUA AAC CTT-3′).

Platination of sense strands and annealing. Platination of the sense strands was performed in aqueous solution with a final oligonucleotide concentration of 2.3 μM and the ratio [oligonucleotide]:[*cis*-$PtCl_2(NH_3)_2$] as 1:1. The platination reaction was carried out in the dark at 37 °C for 8 h, and was then quenched by quick freezing in liquid nitrogen. The obtained samples were kept at −80 °C for no more then 24 h before purification. The platinated products were separated by gel electrophoresis on a 20% denaturing polyacrylamide gel (PAGE). The products were visualized by UV-shadowing, excised and eluted by soaking in 1 M NaOAc (Riedel-de Haen) over night, followed by ethanol precipitation. The siRNAs were annealed in either phosphate buffer (see above) or 1× MOPS buffer by heating 50 μM sense and 50 μM antisense strands for 5 min at 90 °C, followed by slow cooling to room temperature over at least 20 min. The concentrations of the individual strands were determined by their respective absorbance at 260 nm using calculated extinction coefficients based on the nearest-neighbor approximation [22].

Thermal melting of siRNA. Thermal melting studies were performed in 1× MOPS on a Cary 4000 UV-Vis spectrophotometer (Varian) equipped with a temperature control unit. The data was analyzed by use of an online Cary WIN-UV software. The concentration of unplatinated siRNA was varied between 0.025 μM and 1.5 μM siRNA, while the concentration of platinated W-siRNA was kept constant at 0.6 μM (duplex concentration). Prior to the melting analysis, the siRNAs were annealed by heating to 90 °C for 5 min followed by cooling to 20 °C at a rate of 3 °C/min. Data were collected from 20 °C to 95 °C at a rate of 0.2 °C/min, with intervals of 0.5 °C and a data average time of 2 s. The results were analyzed by both 1st derivative- and hyperchromicity calculations. The ΔG-values were obtained using the van't Hoff equation.

In vitro protein expression in RRL. In vitro protein expression was performed using the T7 Rabbit Reticulocyte Lysate system (RRL, Promega). The plasmids pcDNA3-Luc and pcDNA3-Luc/W-UTR(1–259) containing the bases 1–259 of the Wnt-5a 3′ UTR were used as templates for protein expression. The plasmids were constructed as previously described [18,21]. Typically, the reactions were performed with 10 ng/μl of the pcDNA3-Luc or pcDNA3-Luc/W-UTR(1–259) plasmids. A renilla plasmid was added to all reactions as an internal reference, also at a concentration of 10 ng/μl. The final reaction volume was 10 μl, and siRNAs were added with a final concentration of 5 μM. In the control lacking siRNA, a similar setup was made adding 1× phosphate buffer instead of siRNA to reach a total volume of 10 μl. After 1.5 h incubation at 30 °C the lysates were tested for Firefly Luciferase and Renilla Luciferase activities with the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's protocol. The reactions were analysed in at least triplicates. The obtained Firefly luciferase luminescence was normalized against the Renilla luciferase luminescence, and the ratio reported as Luciferase activity. The effect on the expression was examined both as a function of siRNA, and as a function of platinated versus non-platinated siRNA.

In vitro protein expression in HB2 cells. The HB2, non-cancerous mammary epithelial cell line was used for the cellular *in vitro* studies. The cell line is a subclone of the MTSV-7 cell line originating and kindly received from the laboratory of Dr. J. Taylor-Papadimitriou (ICRF, UK) [23]. Cells were cultured in normal DMEM at 37 °C and in a humidified atmosphere with 5% carbon dioxide. Transfection was performed in 24 well plates using 50,000 cells per well, 0.4 μg/well pMIR-Luc or pMIR-Luc/W-UTR (1–260), and 0.4 μg/well pMIR-Renilla. Renatured W-siRNA (40 nM) was co-transfected with the plasmids. The medium was changed to normal DMEM after 4 h, and the cells were incubated for 40 h prior to lysis in 100 μl lysis buffer (Promega). The lysed samples were immediately frozen in liquid nitrogen, and stored at −80 °C until analysis. The Luciferase activity was determined as described above.

Results and discussion

Platination of sense siRNA and product distribution

Exposure of the sense strands of W-siRNA33, W-siRNA147, and W-siRNA156 to cisplatin gave rise to one single product only. The resulting double stranded platinated siRNAs are denoted W-siRNA33-Pt, W-siRNA147-Pt, and W-siRNA156-Pt. In the case of W-siRNA139, two distinct products were obtained, W-siRNA139-Pt1 and W-siRNA139-Pt2. The different product distribution patterns can be accounted for by consideration of the number and location of preferred GG- or AGA-platination sites [24–26] present in the respective oligomers. More specifically, for W-siRNA147 and W-siRNA156 only one GG-site is present. In the case of W-siRNA33, the tentative GG and AGA sequences are both located at the end of the oligomer, i.e., equally disfavoured from an electrostatic point of view [27,28], thus allowing formation of the kinetically most favoured product, i.e., platination of GG. In W-siRNA139, the AGA-site is located in the middle of the oligomer whereas the GG-site is located at the 3′ end. Under these circumstances two products are formed, indicating

that the higher tendency for pre-accumulation of the platinum complex in the middle of the oligomer is enough to compensate for the higher inherent reactivity exhibited by the GG-site [29].

Influence of platination on thermal stability of W-siRNA

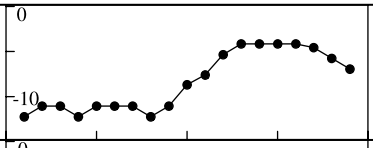
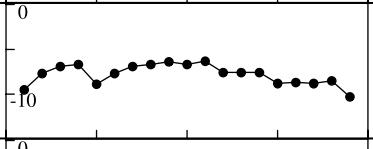
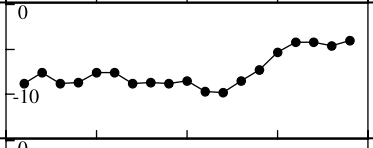
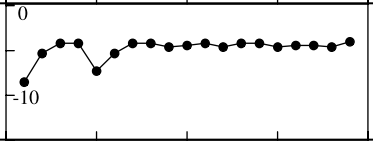
A summary of the melting temperatures and related thermodynamic parameters obtained for the non-platinated

Table 1
Thermodynamic parameters for platinated and non platinated siRNAs; W-siRNA33, W-siRNA33-Pt, W-siRNA139, W-siRNA139-Pt1, W-siRNA139-Pt2, W-siRNA147, W-siRNA147-Pt, W-siRNA156 and W-siRNA156-Pt, all obtained in 1× MOPS buffer

siRNA	$T_{m,\ deriv}$[a]/°C	$T_{m,\ hypo}$[b]/°C	ΔH/kJ mol^{-1}	ΔS/J mol^{-1}K^{-1}	ΔG_{20}/kJ mol^{-1}	ΔG_{25}/kJ mol^{-1}
W-siRNA33	76	74	−463	−1184	−116	−110
W-siRNA33-Pt	72	71	−410	−1051	−102	−97
W-siRNA139	75	73	−675	−1811	−144	−135
W-siRNA139-Pt-1	72	70	−438	−1151	−100	−94
W-siRNA139-Pt-2	72	70	−398	−1038	−99	−94
W-siRNA147	65	63	−589	−1627	−112	−104
W-siRNA147-Pt	55	54	−463	−1292	−85	−78
W-siRNA156	53	52	−673	−1947	−101	−92
W-siRNA156-Pt	50	50	−575	−1658	−85	−77

[a] Melting points determined from 1st derivative of absorbance vs. temperature profile.
[b] Melting point determined from hyperchromicity measurements.

Table 2
Schematic overview of the siRNAs used and related properties

siRNA	Sequence[a]	Internal base stability[b] ΔG (kcal/mol)	Pt-sites[c] GG	Pt-sites[c] AG or GA	# products[d]	T_m (°C)[e] 1M NaCl	T_m (°C)[e] 1×MOPS	T_m (°C)[e] 1×MOPS	Suppression in RRL[f] siRNA	Suppression in RRL[f] siRNA-Pt
W-siRNA33	$^{5'}$GGACCCGCUUAUUUAUAGATT$^{3'}$ $_{3'}$TTCCUGGGCGAAUAAAUAUCU$_{5'}$		1	3	1	79	74	73	19	49
W-siRNA139	$^{5'}$CCAUCUAAGAACUCUGUGGTT$^{3'}$ $_{3'}$TTGGUAGAUUCUUGAGACACC$_{5'}$		1	2	2	79	75	72	26	7
W-siRNA147	$^{5'}$GAACUCUGUGGUUUAUUAUTT$^{3'}$ $_{3'}$TTCUUGAGACACCAAAUAAUA$_{5'}$		1	1	1	72	65	55	30	25
W-siRNA156	$^{5'}$GGUUUAUUAUUAAUAUUAUTT$^{3'}$ $_{3'}$TTCCAAAUAAUAAUUAUAAUA$_{5'}$		1	0	1	60	53	50	32	32

[a] The column stating the sequence displays the sense strand, 5′- to 3′ end, on top of the antisense strand, 3′- to 5′ end.
[b] The internal stability (ΔG (kcal/mol)) was determined from sfold [33] and displayed from the 5′ position of the sense strand.
[c] Potential Pt(II) binding sites: Number of GG and AG- or GA sites in the sense strand.
[d] Number of platinated products: The number of product bands that were visible on a PAA gel by UV shaddowing, following platination.
[e] T_m: The first two columns give T_m for unplatinated siRNA, the third is W-siRNA platinated in the sense strand. Suppression in RRL:The plasmids pcDNA3-Luc/W-UTR(1–259) or pcDNA3-Luc were incubated together with 5 μM WsiRNA, in RRL for 90 min at 30 °C.
[f] Values refer to Luciferase activity obtained with pcDNA3-Luc/W-UTR(1–259) normalized to the Luciferase activity obtained with pcDNA3-Luc in percent.

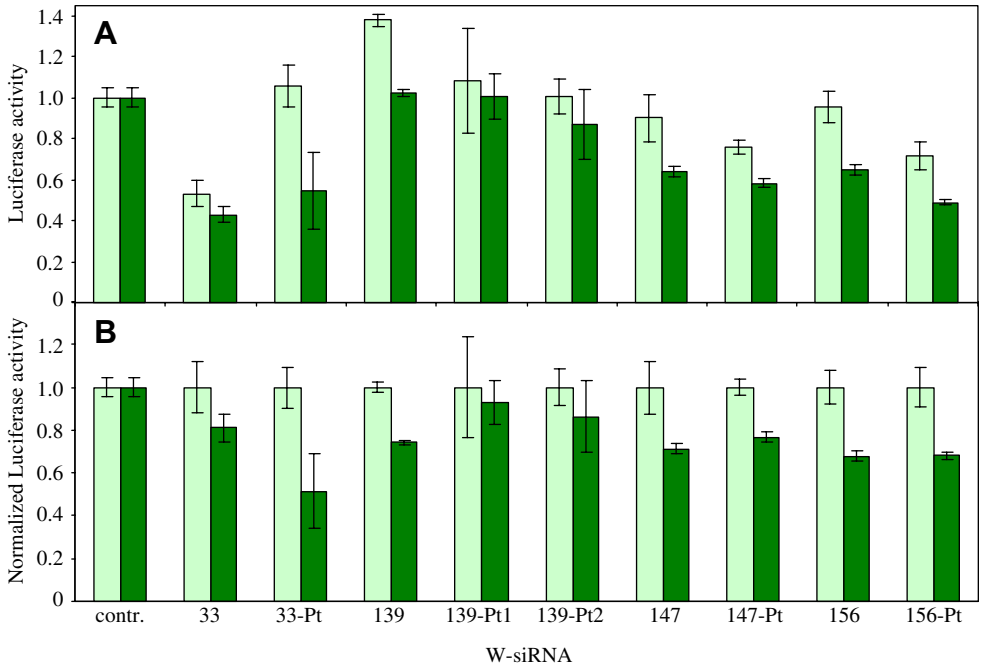


Fig. 1. (A) Luciferase activity and (B) normalized luciferase activity obtained after incubation of pcDNA3-Luc (light green), or pcDNA3-Luc-L/W-UTR(1–260) (dark green) for 90 min at 30 °C in RRL with W-siRNA. The plasmids were incubated in the absence (contr.) and presence of 5 μM W-siRNA33 (33), W-siRNA33-Pt (33-Pt), W-siRNA139 (139), W-siRNA139-Pt1 (139-Pt1), W-siRNA139-Pt2 (139-Pt2), W- siRNA147 (147), W- siRNA147-Pt (147-Pt), W-siRNA156 (156), or W-siRNA156-Pt (156-Pt). Buffer at pH 6.5 was used as control (contr.).

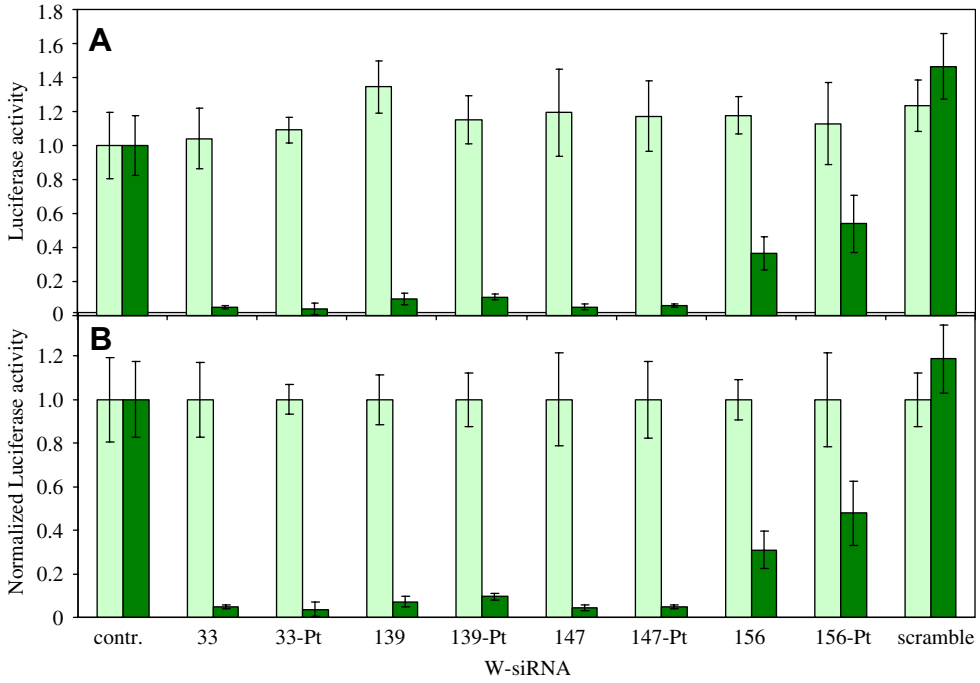


Fig. 2. (A) Luciferase activity and (B) normalized luciferase activity obtained after transfection of HB2 cells with pMIR-Luc (light green), or pMIR-Luc/W-UTR(1–260) (dark green). The plasmids were incubated in the absence (contr.) and presence of 40 nM W-siRNA33 (33), W-siRNA33-Pt (33-Pt), W-siRNA139 (139), W-siRNA139-Pt (139-Pt), W-siRNA147 (147), W- siRNA147-Pt (147-Pt), W-siRNA156 (156), W-siRNA156-Pt (156-Pt) or scramble siRNA.

siRNAs, together with the corresponding platinated ones is made in Table 1. As can be seen in the table, the melting temperatures ($T_{m,\ deriv}$) determined for the non-platinated siRNAs vary in the range 50– 75 °C, and agree well with the corresponding theoretically predicted ones [30]. After platination, the T_m-values are decreased by 3–10 °C.

As judged by the thermodynamic parameters, the decrease in T_m is a combined result of an increase in both ΔH and ΔS, i.e., due to both destabilization of the duplex formation and an increase of entropy, all in agreement with the structural change and loss of hydration that can be expected after adduct formation with the positively charged platinum center [31].

Interference of W-siRNA and W-siRNA-Pt with protein translation in RRL and HB2

The four different siRNAs investigated in the present study were all designed to target the conserved AU-rich region containing a putative HuR binding site [18]. With respect to internal thermodynamic base stability profile, W-siRNA33, W-siRNA147 and W-siRNA156 were designed with a high-melting region at the 5′ end of the sense strand, compare also Table 2. However, only modest differences between the translational inhibitory efficacies of the unplatinated siRNAs are observed in RRL. The down regulation of protein expression lies between 20 and 40% with W-siRNA147 and W-siRNA156 as the most potent siRNAs, and W-siRNA33 as the least efficient one, see Fig. 1. After platination of the sense strand, a different picture is obtained however. The most noticable difference is observed for W-siRNA33-Pt which reaches a suppression level around 50%, i.e., an improvement of the efficacy by more than 100% compared with the corresponding unplatinated W-siRNA33. As judged by primary fluorescence data (data not shown), the effect seems likely to be due to suppression of off-target effects caused by the sense strand. We therefore speculate that the presence of the platinated site on the siRNA sense strand serves as a block for loading onto RISC, thus can be utilized as a method for elimination of off-target effects. For the other three siRNAs, the introduction of the platinated sense strand has only marginal effect on the translational inhibition. In the case of W-siRNA139-Pt1 and W-siRNA139-Pt2, the platination leads to a somewhat less efficient siRNA. The observation suggests that the thermal destabilization caused by the attached platinum compound, when located both in the middle of the siRNA and close to the 3′ end of the sense strand, interferes with processing of the siRNA.

By changing the test environment from the RRL system to the HB2 cell line the sensitivity towards added siRNA is increased by 2 to 3 orders of magnitude, i.e., showing significant suppression levels in the nano-molar range, see Fig. 2. With exception of W-siRNA156, all siRNAs exhibit suppression levels well above 80%, both as unplatinated and platinated W-siRNA. Interestingly, W-siRNA156 which partially overlaps with a tentative miRNA target region [32], is the least efficient one. The lower efficacy of W-siRNA156 shows that this particular region is stabilized in the cellular environment and less prone to siRNA induced degradation. The observation is well in agreement with previous reports suggesting proteins such as HuR to be responsible for stabilization of the Wnt-5a mRNA [18]. It might thus be possible that the high efficacy obtained for the other siRNAs could be a combined effect of destabilization of the HuR binding motif and siRNA induced degradation. Further studies are however needed to clearly elucidate the cellular mechanisms responsible for the large variations in efficacy between the closely related W-siRNAs studied in the present work.

Acknowledgments

Final support from Cancerfonden, Vetenskapsrådet, Kgl. Fysiografiska Sällskapet i Lund, Crafoordska Stiftelsen, and FLÄK (Research School in Medicinal Science at Lund U) is gratefully acknowledged. We are grateful for the kind gift of pcDNA3 plasmids from K. Leandersson and the HB2 cell line from J. Taylor-Papadimitriou.

References

[1] H. Herweijer, J.A. Wolff, Gene therapy progress and prospects: hydrodynamic gene delivery, Gene Therapy 14 (2007) 99–107.
[2] D. Cejka, D. Losert, V. Wacheck, Short interfering RNA (siRNA): tool for therapeutic? Clin. Sci. 110 (2006) 47–58.
[3] G. Tang, siRNA and miRNA: an insight into RISCs, Trends Biochem. Sci. 30 (2005) 106–114.
[4] F. Eckstein, Small non-coding RNAs as magic bullets, Trends Biochem. Sci. 30 (2005) 445–452.
[5] Y. Tomari, C. Matranga, B. Haley, N. Martinez, P.D. Zamore, A Protein Sensor for siRNA Asymmetry, Science 306 (2004) 1377–1380.
[6] I. Ladunga, More complete gene silencing by fewer siRNAs: transparent optimized design and biophysical signature, Nucl. Acids Res. 35 (2007) 433–440.
[7] S. Schubert, A. Gruenweller, V.A. Erdmann, J. Kurreck, Local RNA target structure influences siRNA efficacy: systematic analysis of intentionally designed binding regions, J. Mol. Biol. 348 (2005) 883–893.
[8] K.M. Brown, C.-Y. Chu, T.M. Rana, Target accessibility dictates the potency of human RISC, Nat. Str. Mol. Biol. 12 (2005) 469–470.
[9] A. Khvorova, A. Reynolds, S.D. Jayasena, Functional siRNAs and miRNAs exhibit strand bias, Cell 115 (2003) 209–216.
[10] E. Martin Scott, J. Caplen Natasha, Mismatched siRNAs downregulate mRNAs as a function of target site location, FEBS Lett. 580 (2006) 3694–3698.
[11] P.D. Zamore, Methods and compositions for enhancing efficacy and specificity of single and double blunt-ended siRNA by asymmetrically weakening base pair interactions at one end, (University of Massachusetts, USA). Application: WOWO (2005) p. 44.
[12] Q. Du, H. Thonberg, J. Wang, C. Wahlestedt, Z. Liang, A systematic analysis of the silencing effects of an active siRNA at all single-nucleotide mismatched target sites, Nucl. Acids Res. 33 (2005) 1671–1677.
[13] T. Holen, S.E. Moe, J.G. Sorbo, T.J. Meza, O.P. Ottersen, A. Klungland, Tolerated wobble mutations in siRNAs decrease specificity, but can enhance activity *in vivo*, Nucl. Acids Res. 33 (2005) 4704–4710.
[14] B. Zhang, Q. Wang, X. Pan, MicroRNAs and their regulatory roles in animals and plants, J. Cell Physiol. 210 (2007) 279–289.
[15] G.A. Calin, C.M. Croce, MicroRNA signatures in human cancers, Nature Rev. Cancer 6 (2006) 857–866.
[16] K.G. Danielson, J. Pillarisetti, I.R. Cohen, B. Sholehvar, K. Huebner, L.-J. Ng, J.M. Nicholls, K.S.E. Cheah, R. Iozzo, Characterization of the complete genomic structure of the human WNT-5A gene, functional analysis of its promoter, chromosomal mapping, and expression in early human embryogenesis, J. Biol. Chem. 270 (1995) 31225–31234.

[17] C.C. Clark, I. Cohen, I. Eichstetter, L.A. Cannizzaro, J.D. McPherson, J.J. Wasmuth, R.V. Lozzo, Molecular cloning of the human proto-oncogene Wnt-5A and mapping of the gene (WNT5A) to chromosome 3p14-p21, Genomics 18 (1993) 249–260.
[18] K. Leandersson, K. Riesbeck, T. Andersson, Wnt-5a mRNA translation is suppressed by the Elav-like protein HuR in human breast epithelial cells, Nucl. Acids Res. u34 (2006) 3988–3999.
[19] C.-Y.A. Chen, N. Xu, A.-B. Shyu, Highly selective actions of HuR in antagonizing AU-rich element-mediated mRNA destabilization, Mol. Cell Biol. 22 (2002) 7268–7278.
[20] C. Barreau, L. Paillard, H.B. Osborne, AU-rich elements and associated factors: are there unifying principles? Nucl. Acids Res. 33 (2005) 7138–7150.
[21] M. Hägerlöf, P. Papsai, H. Hedman, U. Jungwirth, V. Jenei, S.K.C. Elmroth, Manuscript in preparation.
[22] E.G. Richards, Use of tables in calculation of absorption, optical rotatory dispersion and circular dichroism of polyribonucleotides, thrird ed., in: G.D. Fasman (Ed.), Handbook of Biochemistry and Molecular Biology, vol. 1, CRC Press, Cleveland, OH, USA, 1975, pp. 596–603.
[23] J. Bartek, J. Bartkova, N. Kyprianou, E.N. Lalani, Z. Staskova, M. Shearer, S. Chang, J. Taylor-Papadimitriou, Efficient immortalization of luminal epithelial cells from human mammary gland by introduction of simian virus 40 large tumor antigen with a recombinant retrovirus, Proc. Natl. Acad. Sci. USA 88 (1991) 3520–3524.
[24] A.M.J. Fichtinger-Schepman, P.H.M. Lohman, J. Reedijk, Detection and quantification of adducts formed upon interaction of diamminedichloroplatinum(II) with DNA by anion-exchange chromatography after enzymic degradation, Nucl. Acids Res. 10 (1982) 5345–5356.
[25] V. Monjardet-Bas, M.-A. Elizondo-Riojas, J.-C. Chottard, J. Kozelka, A combined effect of molecular electrostatic potential and guanine N7 accessibility explains sequence-dependent binding of *cis*-[Pt(NH3)2(H2O)2]2+ to DNA duplexes, Angewandte Chemie, International Edition 41 (2002) 2998–3001.
[26] M.S. Davies, S.J. Berners-Price, T.W. Hambley, Rates of platination of AG and GA containing double-stranded oligonucleotides: insights into why cisplatin binds to GG and AG but not GA sequences in DNA, J. Am. Chem. Soc. 120 (1998) 11380–11390.
[27] J. Kjellstroem, S.K.C. Elmroth, Medium effects on reactivity profiles for platination of phosphorothioate-containing oligonucleotides, Inorg. Chem. 38 (1999) 6193–6199.
[28] A. Sykfont, A. Ericson, S.K.C. Elmroth, Non-uniform rate for platination of guanine-N7 located in short DNA oligomers, Chem. Commun. (2001) 1190–1191.
[29] A.H. Elcock, J.A. McCammon, Sequence dependent hydration of DNA: theoretical results, J. Am. Chem. Soc. 117 (1995) 10161–10162.
[30] M. Zuker, Mfold web server for nucleic acid folding and hybridization prediction, Nucl. Acids Res. 31 (2003) 3406–3415.
[31] P.M. Takahara, C.A. Frederick, S.J. Lippard, Crystal structure of the anticancer drug cisplatin bound to duplex DNA, J. Am. Chem. Soc. 118 (1996) 12309–12321.
[32] Targetscan3.1: http://www.targetscan.org.
[33] Y. Ding, C.Y. Chan, C.E. Lawrence, Nucl. Acids Res. 32 (2004) W135–W141, Web server issue.