Systematic Evolution of a DNA Aptamer Binding to Rat Brain Tumor Microvessels

SELECTIVE TARGETING OF ENDOTHELIAL REGULATORY PROTEIN PIGPEN

Received for publication, January 16, 2001
Published, JBC Papers in Press, February 13, 2001, DOI 10.1074/jbc.M100347200

Michael Blank‡§, Toni Weinschenk‡, Martin Priemer¶, and Hermann Schluesener‡

From the ‡Institute of Brain Research, University of Tuebingen, Calwer Strasse 3, D-72076 Tuebingen, Germany, the ¶Institute for Cell Biology, Department of Immunology, University of Tuebingen, Auf der Morgenstelle 15, D-72076 Tuebingen, Germany, and the ##Institute for Cell Biology, Department of Molecular Biology, University of Tuebingen, Auf der Morgenstelle 15, D-72076 Tuebingen, Germany

Tumor microvessels differ in structure and metabolic function from normal vasculature, and neoangiogenesis is associated with quantitative and qualitative changes in expression of endothelial proteins. Such molecules could serve as molecular addresses differentiating the tumor vasculature from those of the normal brain. We have applied Systematic Evolution of Ligands by EXponential enrichment (SELEX) against transformed endothelial cells as a complex target to select single-stranded DNA-ligands (aptamers) that function as histological markers to detect microvessels of rat experimental glioma, a fatal brain tumor that is highly vascularized. Both the SELEX selection procedure as well as subsequent deconvolution-SELEX were analyzed by fluorescence based methods (flow cytometry and fluorescence microscopy). Of 25 aptamers analyzed, one aptamer was selected that selectively bound microvessels of rat brain glioblastoma but not the vasculature of the normal rat brain including peritumoral areas. The molecular target protein of aptamer III.1 was isolated from endothelial cells by ligand-mediated magnetic DNA affinity purification. This protein was identified by mass spectrometry as rat homologue of mouse pigpen, a not widely known endothelial protein the expression of which parallels the transition from quiescent to angiogenic phenotypes in vitro. Because neoangiogenesis, the formation of new blood vessels, is a key feature of tumor development, the presented aptamer can be used as a probe to analyze pathological angiogenesis of glioblastoma. The presented data show that pigpen is highly expressed in tumor microvessels of experimental rat brain glioblastoma and may play an important role in warranting blood supply, thus growth of brain tumors.

Neangiogenesis, the new formation of blood vessels is associated with endothelial cell (EC) proliferation, migration, and formation of new capillaries as a response to the increased demand of tumor tissue for oxygen and nutrients (1). In normal physiological processes, such as wound-healing, angiogenesis is tightly balanced by positive and negative regulators. In several disease states, such as tumor growth, overactive angiogenesis contributes to advancement of disease (2, 3). Positive regulators of angiogenesis are recruited by the tumor to dominate negative regulators to ensure proliferation and organization of microvessel-forming EC. Thus, neoangiogenic EC, associated with tumor development, differ from preexisting, quiescent EC by qualitative or quantitative changes of molecular addresses, which could serve as potential targets for tumor homing diagnostic and therapeutic agents (4–6). A series of targets and corresponding antibodies to address the activated endothelium of pathological blood vessels are known (2). But it must be supposed that there are other regulators, so far unknown, to be involved in the complex process of angiogenesis. To define novel molecular addresses of the tumor vasculature, combinatorial chemistry approaches, the libraries of which are not biased by natural mechanisms of selection such as immune tolerance (7), have been successfully applied to develop antibody fragments by phage display that bind to tumor blood vessels (8–12). However, peptides require circularization, dimerization, or presentation in the context of a larger protein for binding to a target (13). These structural requirements limit the number of potential ligands that can be sampled in an experiment directed against complex targets. Systematic Evolution of Ligands by EXponential enrichment (SELEX) is a nucleic acid based combinatorial chemistry procedure that has been used to isolate relatively short high affinity ssDNA or RNA ligands, termed aptamers, to a wide variety of protein or low molecular weight compounds (14, 15). Single-stranded nucleic acids, which can fold into very small and complex three-dimensional shapes with a great diversity of binding specificities, are isolated from a large pool of random sequence molecules (10¹⁴–10¹⁵ sequences) by reiterative rounds of selection and amplification (reviewed in 16).

Here we describe a fluorescence-based SELEX procedure. Using transformed EC as a complex target allowed systematic evolution of fluorescence-labeled oligonucleotides and subsequent in situ deconvolution-SELEX by flow cytometry and fluorescence microscopy on cryostat tissue sections (Fig. 1). Finally aptamers were generated that function as a histological marker to selectively stain microvessels of experimental rat glioblastoma, a brain tumor that is highly vascularized (17). We present aptamer III.1, a ssDNA-ligand binding to pigpen, a
not widely known endothelial protein of the Ewing’s sarcoma family that parallels the transition from quiescent to angiogenic phenotypes in vitro (18).

EXPERIMENTAL PROCEDURES

Cell Lines—Endothelial cell line YPEN-1 (CRL-222) immortalized by an Adenovirus-12 SV40 hybrid virus (19), microglial cell line N9 (20), and rat glioblastoma cell line C6 (21) were obtained from ATCC (American Type Culture Collection). YPEN-1 cells and N9 microglial cells were cultured in RPMI 1640 (Life Technologies, Inc.), supplemented by 10% fetal calf serum (Seromed) to subconfluence, and harvested by carefully dislodging the cells from tissue culture flasks.

SELEX Library and Primers—The high pressure liquid chromatography-purified library contained a central randomized sequence of 60 nucleotides (nt) flanked by 18-nt primer hybridization sites (5′-ATA CCA GCT TAT TCA ATT-3′; MWG-Biotech; G) (22). A fluorescein isothiocyanate (FITC)-labeled 5′-primer (5′-FITC-18C-ATA CCA GCT TAT TCA ATT-3′; MWG-Biotech) was prepared as described above.

Flow Cytometric Analysis of Apatmers Bound to YPEN-1 EC in Successive SELEX Rounds—Specific EC-binding, FITC-labeled aptamers were monitored in the second and subsequent rounds. After incubation of 10^5 EC with 200 pmol aptamer in 200 μl of selection buffer (with a 5-fold molar excess of tRNA and BSA), cells were washed twice in 1 ml of selection buffer (with 0.2% BSA), and FITC fluorescence was monitored by flow cytometry and fluorescence microscopy. FITC-sDNA was prepared as described above.

Deconvolution-SELEX (Step 1): Flow Cytometric Analysis of Individual Apatmers—5 × 10^4 YPEN-1 endothelial cells and N9 microglial cells, respectively, were each preincubated with selection buffer containing 1 μg/ml tRNA for 20 min on ice and incubated with 200 μl of denatured and renatured FITC-aptamer solution (0.25 pmol/μl with 1 μg/ml tRNA) for 30 min at 37 °C. Cells were washed twice in selection buffer (with 0.2% BSA), and fluorescence was determined by flow cytometry.

Deconvolution-SELEX (Step 2): Preparation of Rat Brain Glioblastoma Tissue Sections and Histological Analysis—2–2.5 × 10^6 tumor cells from a glioblastoma cell line were transplanted intracranially into Harlan Sprague-Dawley rats as described (21). Rats were perfused 23 days after transplantation with phosphate-buffered saline, and brains were removed for cryostat sectioning. Binding of individual FITC-aptamers was analyzed on rat cryostat sections of normal and glioblastoma brain. Tissue sections (10 μm) were preincubated with selection buffer (with 1 μg/ml tRNA) at 4 °C for 20 min and then incubated with FITC-aptamer solution (0.25 pmol/μl with 1 μg/ml yeast tRNA) at room temperature.
temperature for 40 min. Tissue sections were washed twice in selection buffer (with 0.2% BSA), and fluorescence patterns were analyzed microscopically. For double labeling, cryostat tissue sections of rat brain glioblastoma were stained with aptamer III.1 as described above and subsequently stained with 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) (100 ng/ml) or mouse CD-31 (1 h at a dilution of 1/1000; Dako) with C57H5 anti-mouse secondary IgG (30 min at a dilution of 1/200; Amersham Pharmacia Biotech), respectively. After two washes in selection buffer (with 0.2% BSA), fluorescence patterns were analyzed microscopically.

Wounding experiments were performed in Petri dishes. The confluent monolayer of YPEN-1 EC was wounded by carefully ditching cells with a sterile pipette tip. The cells in Petri dishes were cultured for an additional 4 h, washed twice with selection buffer, and subsequently fixed with methanol (10 min, −20 °C; Merck). After incubation of the cell layer with FITTC-18C-aptamer III.1 (0.25 pmol/μl with 1 μg/μl tRNA) in selection buffer (30 min, 37 °C), staining patterns were analyzed by fluorescence microscopy.

RESULTS

Selection of ssDNA Aptamers that Bind Transformed Endothelial Cells—SELEX was used essentially as described (30) to generate ssDNA aptamers against Adenovirus-12 SV40-transformed YPEN-1 rat endothelial cells. Commonly used radioac-
tive labeling of nucleic acids (to monitor the enrichment of aptamers in successive SELEX rounds) was replaced by attaching FITC as a fluorescence reporter molecule. The selection was initiated with a SELEX library of ~1 × 10^{15} sequences, each containing a central randomized region of 60 nt flanked by two primer hybridization sites (22). To reduce co-selection of aptamers binding to other cell types, counterselection against N9 microglial cells (20), a population of brain monocytes, was performed prior to each selection round. To monitor the enrichment of specific cell-binding aptamers during selection, SELEX pools of the second and following rounds were analyzed by flow cytometry after incubation with YPEN-1 endothelial cells (Fig. 2). Analysis of fluorescence-labeled pools in successive cycles of selection showed a pronounced shift of second round histogram toward higher fluorescence intensity. The histograms of SELEX pools 3–7 showed slow but steady increases in fluorescence intensity and thus enrichment of endothelial cell-binding aptamers.

Deconvolution-SELEX—As the utility of a selected pool of nucleic acids to dissect complex systems is contingent upon the ability to quickly identify which sequences are ligands for components of interest within the targeted mixture (30), individual aptamers cloned from the eighth round of selection were evaluated by a two step deconvolution-SELEX procedure: (i) each sequence affinity against the transformed YPEN-1 EC cell line was reevaluated by flow cytometry, and (ii) the selective binding of aptamers to microvessels of C6 brain tumor was analyzed by fluorescence microscopy on cryostat tissue sections of rat brain glioblastoma. Of 25 sequences (FITTC-18C-96-nt) tested by flow cytometry, by comparison of endothelial cells stained with unselected ssDNA (FITTC-18C-96-nt), 23 showed binding to YPEN-1 endothelial cells. Of these, 16 bound to the pathological microvasculature of C6 glioblastoma, and 7 showed no binding. Aptamer III.1 displayed the most intensive staining of the vasculature exclusively in areas of solid tumor growth and was therefore chosen as the candidate to be further characterized as outlined below.

Increased fluorescence intensity of YPEN-1 EC stained with fluorescence-labeled aptamer III.1 (sequence and proposed secondary structure is illustrated in Fig. 3C) by comparison with unselected ssDNA (FITTC-18C-96-nt) as a negative control is shown in fluorescence-activated cell sorter histograms of Fig. 3A. To demonstrate also EC-specific binding by flow cytometry, binding analyzes against N9 microglial cells was performed (Fig. 3B). Histological analyzes of aptamer III.1 is illustrated in Fig. 4. FITC-labeled aptamer stained the complex architecture of the pathological microvasculature (Fig. 4A). Counterstaining of cellular nuclei with DAPI (blue) demonstrated selective targeting...
A DNA Aptamer Binding Brain Tumor Microvessels

Fig. 3. Cell binding of FITC-18C modified aptamers. Red curves, aptamer III.1. Black curves, unselected ssDNA (FITC-18C-96-nt). A, aptamer III.1 shows specific binding to transformed rat endothelial cells but not to B, N-9 microglial cells. C, proposed secondary structure of aptamer III.1 (FITC-18C-ATAACCAGCTTATTCAATTAGGGCCTGGC-ATTGTGGTTGAGTATATAGTTAGGAGGTTGTTGAGACTAGTCGCA-AGATATAGATAGTAGTAAGTGCAATCT; primer hybridization sites are underlined).

Fig. 4. Staining of cryostat sections of rat brain glioblastoma. A, FITC-18C-conjugated aptamer III.1 shows selective staining of microvessels embedded in their complex surrounding of glioblastoma. B, counterstaining of cellular nuclei with DAPI (blue) visualizes selective labeling of microvessels with aptamer III.1 in the cell-rich tumor region but not of vessels in peritumoral areas. C, staining with CD-51 (red) and unselected SELEX genome (FITC-18C-96-nt) shows no double labeling of endothelial cells or vessel-associated structures. D, double staining with endothelial CD-51 mAb (CyTM3, red) III.1 countersigns aptamer III.1 positive cells (FITC, green) as endothelial cells. E, staining of Ypen-1 endothelial cell culture after wounding a confluent cell layer with a pipette tip. Aptamer III.1 (green) shows significantly increased binding to endothelial cells in the subconfluent area. (scale bars, 50 μm).

Fig. 5. Coomassie Blue-stained polyacrylamide gel used to analyze the ligand-mediated target purification. Lane A, molecular marker. Lane B, purification with aptamer III.1. Lane C, purification with unselected ssDNA (trB-96-nt). Lane D, untreated endothelial cells. The target band isolated in the reaction containing the aptamer III.1 is indicated by an asterisk and was identified as the 67-kDa protein pigpen.

of microvessels within the cell-rich tumor region but not of vasculature in peritumoral areas (Fig. 4B). Fluorescence microscopy of rat normal brain after incubation with aptamer III.1 did not show any staining of EC or vessel-associated structures (data not shown). Staining of tumor tissue sections with mouse IgG directed against EC (CD-31, visualized with anti-mouse IgG-CyTM3, red) and subsequent labeling with aptamer III.1 (FITC, green) countersigned the aptamers target as EC (Fig. 4D). Rat brain tumor tissue sections were analyzed after incubation with unselected FITC-18C-ssDNA and endothelial CD-31 (CyTM3, red) as a control. Fig. 4C demonstrates negative binding.

To get further evidence for aptamer III.1 pathological association, cultured EC were analyzed after wounding the confluent cell layer with a pipette tip. Subsequent staining with aptamer III.1 and fluorescence microscopy showed exclusive up-regulation of the molecular target of aptamer III.1 in endothelial cells next to the lesion, a region that is characterized by increased proliferation compared with areas of adjacent contact-inhibited cells of the confluent monolayer (Fig. 4E).

Thus, our histological data gave strong indication that aptamer III.1 binds an endothelial molecular target that is involved in the process of EC proliferation, a key step of angiogenesis.

Aptamer III.1-Mediated Target Identification—The molecular target has been isolated by magnetic DNA affinity purification. Solubilized EC proteins were incubated with trB aptamer III.1 beforehand coupled to magnetic streptavidin beads. After thorough washing and subsequent release of aptamer-bound protein in high salt solution, proteins were analyzed by SDS/polyacrylamide gel electrophoresis (Fig. 5).

Polyacrylamide gel analyses revealed a distinct band migrating at ~70 kDa in the lane of aptamer III.1, which is absent in the lane containing a product of the control reaction (performed with unselected ssDNA) which revealed one single band of ~50 kDa. Peptide mass fingerprinting and sequencing of three of the tryptic peptide fragments of the protein by mass spectrometry and tandem mass spectrometry, respectively, were used to identify the aptamer-specific protein as the rat homologue of mouse pigpen protein (67 kDa), a so far, widely unknown endothelial protein that has been considered to parallel the transition from quiescent to angiogenic phenotypes in vitro (31).

DISCUSSION

Selection by Intact Biological Entities—To select a brain tumor-homing ssDNA ligand we applied SELEX against Adenovirus-12 SV40-transformed YPEN-1 rat endothelial cell line, thus against a pathologic endothelial target for several reasons (32): (i) it can be supposed that in actively growing endothelial cells the balance between positive and negative angiogenic regulators has been shifted toward the side of regulators required for EC proliferation and thus the transformed endothelial cells bear a profile of molecular addresses predominantly expressed by angiogenic tumor microvessels; (ii) the selection by intact biological entities does not require a full understanding of the complex mechanism of EC proliferation, whereas the selection by an isolated protein is limited to cases in which the proteins have been identified and isolated; (iii) because of structural differences in protein conformation, the aptamers...
selected to bind the protein in its purified form will not automatically bind the protein embedded in its natural surrounding; (iv) the selection using intact, activated EC give promises in the identification of components that have not been known for their critical role in angiogenesis, (v) moreover, the cellular target allowed fluorescence-based selection thus to monitor the enrichment of cell-binding, fluorescence-labeled ssDNA-ligands in successive pools of selection and amplification by flow cytometry.

**Deconvolution-SELEX**—The eighth round pool was deconvoluted, and single binding of individual aptamers was quantified by flow cytometry (33–36). Further, screening of these individual aptamers on cryostat brain tissue sections allowed deconvolution for their discriminatory binding to neoangiogenic tumor microvessels but not to vessels of the normal rat brain. Thus histological staining allowed the selection of those aptamer candidates recognizing the differentiation of endothelial cells under **in vivo** conditions of angiogenesis.

These two steps of library deconvolution result in aptamer libraries selectively addressing molecular structures, differentiating neoangiogenic endothelium of the brain. The alignment of the 16 aptamer sequences after eight cycles of systematic evolution and the two subsequent steps of deconvolution did not reveal any obvious binding motifs. This appears to be due to the complexity of the endothelial cell with its high number of potential molecular targets. However, the combination of fluorescence-based SELEX and deconvolution-SELEX allowed identification of aptamer candidates, which perform the desired task to act as a histological marker to selectively stain tumor microvessels.

**Aptamers as Histological Markers**—Aptamers were used as histological markers in combination with chromosomal dyes and conventional antibodies for immunostaining. This demonstrates that aptamers are versatile tools that rival antibodies in diagnostic applications (reviewed in 7). Unlike antibodies, synthetic aptamers can easily be produced with a high degree of accuracy, reproducibility, and purity. Therefore, little or no batch to batch variation is expected in aptamer production. They are not sensitive to temperature and undergo reversible denaturation, thus having a much longer self-life (7). Further, aptamers can easily be conjugated to a variety of reporter molecules, chemotherapeutics, or photosensibilisators at precise locations. Thus aptamers selectively binding to tumor endothelium might be of value not only in the development of tumor-homing diagnostics but also in therapeutics. Thus aptamers can easily be conjugated to a variety of reporter molecules, chemotherapeutics, or photosensibilisators at precise locations. Thus aptamers selectively binding to tumor endothelium might be of value not only in the development of tumor-homing diagnostics but also in therapeutics.

**Complex-SELEX as a Tool to Dissect EC Differentiation**—The pronounced and selective staining of rat brain tumor vessels by aptamer III.1 suggested a selective addressing of a molecule involved in neoangiogenesis. We therefore used this aptamer to purify and identify its binding partner, the rat homologous of mouse endothelial pigpen protein. Previous cell culture experiments (18, 31) demonstrate pigpen to be synthe-sized at barely detectable levels in non-proliferating EC, whereas its expression is up-regulated under conditions where EC are actively migrating and dividing (i.e. in subconfluent or wounded cultures) (18). Our observation that the pigpen molecule is highly up-regulated in angiogenic microvessels suggests pigpen as a target for diagnostic imaging or therapeutic strategies in vivo.

The combinatorial chemistry procedure SELEX, applied to select ssDNA ligands against transformed EC as intact biological entities followed by deconvolution-SELEX and aptamer-mediated target identification, has demonstrated its potential to identify compounds that are known or have not been supposed in probably playing a key role in complex mechanisms like angiogenesis (30, 32). In general, generation of fluorescence-labeled, cell-binding aptamers by SELEX opens new avenues in the study of cellular differentiation and tumor diagnostics.

**REFERENCES**

1. Folkman, J. (1995) *Nat. Med.* 1, 27–31
2. Brower V. (1999) *Nat. Biotechnol.* 17, 963–968
3. Zetter, B. R. (1997) *Nat. Biotechnol.* 15, 1243–1244
4. Lund, E. L., Spang-Thomsen M., Skovgaard-Poulsen H., and Kristjansson, P. E. G. (1998) *Acta Neurol. Scand.* 97, 52–62
5. Risau, W. (1997) *Nature* 386, 671–674
6. Thorpe, P. E., and Derhyshire E. J. (1997) *J. Control. Release* 48, 277–288
7. Jayasena S. D. (1999) *Clin. Chem.* 45, 1628–1650
8. Neri, D., Carnemolla, B., Nissim, A., Leprini, A., Querzer, G., Balza, E., Pini, A., Tarli, L., Halin, C., Neri, P., Zardi, L., and Winter, G. (1997) *Nat. Biotechnol.* 15, 1271–1275
9. Arap, W., Pasqualini R., and Ruoslahti E. (1998) *Science* 279, 377–380
10. Koivunen, E., Arap, W., Valtanian, H., Rainiaiso, A., Medina, O. P., Heikkila, P., Kantor, C., Gahenberg, C. G., Salo, T., Kontinen, V. T., Sorera, T., Ruoslahti, E., and Pasqualini, R. (1999) *Nat. Biotechnol.* 17, 768–774
11. Viti, F., Tarli L., Giovannini L., Zardi L., and Neri D. (1999) *Cancer Res.* 59, 347–352
12. Birchler, B., Viti, F., Zardi, L., Spiess, B., and Neri, D. (1999) *Nat. Biotechnol.* 17, 984–988
13. Livnah, O., Stura, E. A., Johnson, D. L., Middleton, S. A., Mulcahy, L. S., Wrighton, N. C., Dower, W. J., Jolliffe, L. K., and Wilson, I. A. (1996) *Science* 273, 464–471
14. Tuerk, C., and Gold L. (1990) *Science* 249, 505–510
15. Ellington, A., and Szostak, J. (1990) *Nature* 346, 505–511
16. Gold, L., Polisky B., Uhlenbeck O., and Yarus M. (1999) *Annu. Rev. Biochem.* 64, 763–797
17. Plate H. P. (1999) *J. Neurosci.* 19, 288–297
18. Alloggio M. C., Alloggio M. A. (1996) *Dev. Biol.* 174, 288–297
19. Yamazaki, K., Lehr J. E., Rhim J. S., and Pienta K. J. (1995) *In Vivo (ATTIK)* 9, 421–426
20. Ferrari, D. (1996) *J. Immunol.* 156, 1531–1539
21. Lampson, L. A., Wen P., Roman V. A., Morri J. H., and Sarid J. A. (1992) *Cancer Res.* 52, 1018–1025
22. Cramer, A., and Stemmer W. P. C. (1993) *Nucleic Acids Res.* 21, 4419
23. Brown, T. A. *DNA Sequencing: The Basics*, IRL Press at Oxford University Press, Oxford
24. Conrad, R. C., Giver L., Tian Y., and Ellington A. D. (1996) *Methods Enzymol.* 267, 336–367
25. Fitzwater, T., and Polisky B. A. (1996) *Methods Enzymol.* 267, 275–301
26. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual 2nd Ed.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
27. Klöcker, N., Kerner, P., Gleichmann, M., Weller, M., and Bahr, M. (1999) *J. Neurosci.* 19, 8517–8527
28. Shevchenko, A., Wilm, M., Vorm, O., and Mann, M. (1996) *Anal. Chem.* 68, 850–858
29. Perkins, D. N., Creasy D. M., and Cottrell, J. S. (1999) *Electrophoresis* 20, 3551–3567
30. Morris, K., Jensen, K. B., Julin, C. M., Weil, M., and Gold, L. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95 2902–2907
31. Alloggio, C. M. (2000) *Exp. Cell. Res.* 255, 270–277
32. Pan, W. P., Craven, R. C., Qui, Q., Wilson, C. B., Wills, J. W., Golovine, S., and Wang, J. F. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 11509–11513
33. Koch, C. J., Evans, S. M., and Lord, E. M. (1995) *Br. J. Cancer.* 72, 869–874
34. Davis, K. A., Abrams B., Lin Y., and Jayasena S. D. (1996) *Nucleic Acids Res.* 24, 752–756
35. Hicke, B. J., Watson, S. R., Konig, A., Lynott, C. K., Bargatzke, R. F., Chang, Y. F., Rinquist, S., Moon-McDonald, L., Jennings, S., Fitzwater, T., Han, H. L., Varki, N., Albinana, I., Willis, M. C., Varki, A., and Parma, D. (1996) *J. Clin. Invest.* 98, 2688–2692
36. Davis, K. A., Lin Y., Abrams B., and Jayasena S. D. (1998) *Nucleic Acids Res.* 26, 3915–3924
Systematic Evolution of a DNA Aptamer Binding to Rat Brain Tumor Microvessels: SELECTIVE TARGETING OF ENDOTHELIAL REGULATORY PROTEIN PIGPEN

Michael Blank, Toni Weinschenk, Martin Priemer and Hermann Schluesener

J. Biol. Chem. 2001, 276:16464-16468.
doi: 10.1074/jbc.M100347200 originally published online February 13, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M100347200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 9 of which can be accessed free at http://www.jbc.org/content/276/19/16464.full.html#ref-list-1