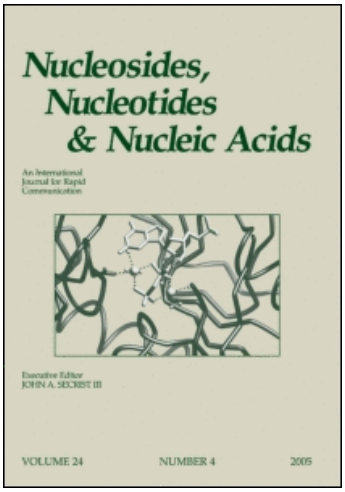

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E. M. Bucci[ab]; M. Valente[a]; D. Musumeci[c]; R. Sapio[a]; V. Anrò[a]; G. N. Roviello[c]; M. Moccia[c]
[a] Bionucleon Srl, Colleretto Giacosa (TO), Italy [b] Bioindustry Park del Canavese, Colleretto Giacosa (TO), Italy [c] Istituto di Biostrutture e Bioimmagini, CNR, Napoli, Italy

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ODN-BASED DRUGS FOR TARGETING OF EXTRACELLULAR PROTEINS

E. M. Bucci □ *Bionucleon Srl, Colleretto Giacosa (TO), Italy and Bioindustry Park del Canavese, Colleretto Giacosa (TO), Italy*

M. Valente □ *Bionucleon Srl, Colleretto Giacosa (TO), Italy*

D. Musumeci □ *Istituto di Biostrutture e Bioimmagini, CNR, Napoli, Italy*

R. Sapio and V. Anrò □ *Bionucleon Srl, Colleretto Giacosa (TO), Italy*

G. N. Roviello and M. Moccia □ *Istituto di Biostrutture e Bioimmagini, CNR, Napoli, Italy*

□ *In this work a novel approach to identify new therapeutic targets consisting of serum proteins which contain an oligonucleotide binding domain is presented.*

Keywords Extracellular proteins; oligonucleotide-based drugs; target validation

INTRODUCTION

For many years oligonucleotides (ODNs) have received considerable attention as tools for selective regulation of gene expression since they provide a rational way to design sequence-specific ligands of nucleic acids (antisense/antigene approach)[1] or DNA-binding regulatory proteins (*decoy* approach).[2] Both for the antisense/antigene and *decoy* strategies, the target has generally to be reached inside the cell, so that intracellular delivery of the therapeutic oligonucleotides is required. Here we show how new therapeutic targets for ODNs, consisting of serum proteins that contain at least one nucleic acid binding domain, may be found outside the cell, avoiding the difficult task of oligonucleotide intracellular delivery.

Address correspondence to E. M. Bucci, Bioindustry Park del Canavese, via Ribes 5, Colleretto Giascosa (TO) 10010, Italy. E-mail: bucci@bioindustrypark.it

RESULTS AND DISCUSSION

The major aim of selecting extracellular targets is to overcome ODN delivery problems. An example of a database that includes extracellular proteins, is the Plasma Proteome Database maintained by the Pandey Lab and by the Institute of Bioinformatic (www.plasmaproteomedatabase.org). When we started our study, this database contained information about 7,518 proteins found in plasma from 3,778 different genes with a rich hand-made annotation. Moreover, each protein in the database is annotated for the presence of the sequence signatures corresponding to the "SMART 5" domain definition, so that the entire database can be screened very easily for the presence of proteins containing a defined domain. By using the signatures corresponding to known nucleic acid binding domains, we identified about 400 proteins, with 45 different nucleic acid binding domains represented. About 25% percent of the identified proteins contain a RNA binding domain, 50% a DNA binding domain and the rest of the proteins have a generic affinity for both DNA and RNA (Figure 1).

Many sequences identified by our procedure contain more than a single nucleic acid binding domain. Moreover, we noticed that among the identified proteins, those with a known extracellular role are very often involved in immunological processes, including inflammation, cellular migration, and immunological aspects of cancer.

The HMG box is an example of DNA binding domain represented in serum proteins, and consists of three alpha helices connected by short loops and is represented in many nuclear proteins involved in the architecture and reorganization of chromatin. When screening for extracellular proteins containing the HMG box, a number of extracellular factors emerged, including HMGB1, a protein that was already under scrutiny by our group as a therapeutic target. HMGB1 is a DNA-binding nuclear protein (Figure 2) that has been recently recognized as a cytokine involved in

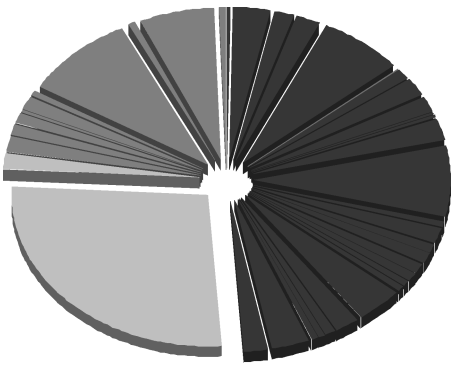

FIGURE 1 Subdivision of ODN-binding serum proteins into RNA binding proteins (dark gray), DNA binding proteins (black), and DNA/RNA binding proteins (light gray).

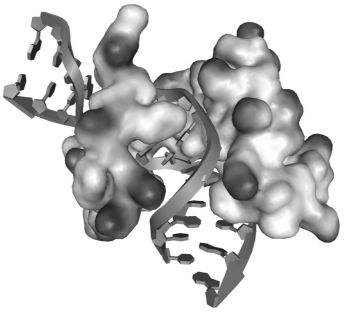

FIGURE 2 Interaction of the HMGB1 homolog HP6A with a DNA duplex, sharply bent by the protein.

a number of different inflammatory diseases.[3] Binding of oligonucleotide-based molecules to these proteins also is a particularly attractive goal, since it has been shown that the HMG box, the recognition site for oligonucleotides, also is required for the cytokine activity of the protein. On the basis of these considerations, we have recently shown that short oligonucleotidic duplexes, properly designed, were able to block HMGB1 cytokine activity in vitro.[4]

The example of HMGB1 shows that, once the target of interest has been identified, the task of designing a ligand based on oligonucleotides is greatly simplified without recurring to SELEX procedures. Furthermore, due to the exracellular location of the target, the conjugation of the ligand to cellular delivery systems is not required; however, the ligand should be resistant to serum nucleases. Moreover, the risk of recognizing undesired complementary ODN sequences is greatly reduced, since the designed ligand has to work extracellularly, avoiding dangerous off-target effects. Once the target protein has been properly selected, by this approach it is possible to achieve the identification of a new diagnostic too, as well as eventually a new inhibitory molecule.

Keeping in mind the example of HMGB1, successfully examined by our group, we are currently working on other extracellular proteins, involved in a specific disease, as target of oligonucleotidic molecule. However, considering the high presence of oligonucleotide-binding proteins outside the cell, and especially in serum, many antisense agents, now in clinical trials, should in our opinion be reconsidered for their potential effects on these proteins.

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