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Inhibition of the DNA binding by the TCF-1 binding RNA aptamer

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Abstract

The DNA binding architectural protein, TCF, and the transcriptional activator, β-catenin, form a complex that regulates the expression of diverse target genes during early development and carcinogenesis. As an approach to modulating transcription by this complex, we selected an RNA aptamer that binds to the DNA binding domain of TCF-1. The aptamer interfered with the binding of TCF-1 to its specific DNA recognition sequences in vitro and also inhibited DNA binding of cellular TCF-1. We also developed the truncated version of the aptamer for efficient delivery to the cells. Structural analysis of the truncated aptamer revealed that a stem–loop with an internal loop was responsible for the binding to TCF-1. Similar approach may well be applicable to other proteins, especially DNA binding transcription factors, in order to modulate their DNA binding and transcriptional activity in the cells.

Keywords: TCF-1; RNA aptamer; HMG domain; DNA binding

T-cell factor-1 (TCF-1) was originally identified as a T-cell specific transcription factor that bound to specific DNA through its high mobility group-1 (HMG-1) DNA binding domain [1–3]. Even though transgenic and knockout approaches suggested that TCF-1 was likely to be involved in the expansion of T-lymphocytes, exact functions of the TCF-1 protein in T-cell development need to be understood [4–8]. TCF family proteins bind to DNA in a sequence-specific manner and they seem to act as architectural proteins for the assembly of other transcription factors [9]. Identification of β-catenin as a potent transcriptional co-activator of TCF family proteins led to a greater understanding of their function [10,11]. Since it is highly expressed in various cancer cells, it seemed possible that the formation of a transcriptional complex by an oncogenic β-catenin with TCF might be a central event in cancer cell development [12–14]. The TCF/β-catenin protein complex is also a critical regulator of early developmental events such as axis formation in the *Xenopus* embryo and Wingless signaling in *Drosophila* [15–17]. In addition, it was recently shown that the TCF/β-catenin complex mediating Wnt signaling seems to be an important pathway in immature thymocyte development [18–20]. Interestingly, the specific isoform of TCF-1 that bound to β-catenin was the only protein effective in ensuring the survival of immature thymocytes [21]. These findings began to point to the role of TCF family proteins as critical modulators of the expression of genes that control the decision between proliferation and apoptosis [22,23]. For example, TCF/β-catenin transcribes genes implicated in cancer development, such as cyclin D1 and c-myc [24,25].

A specific tool for modulating such transcription, either by inhibiting DNA binding or by disrupting protein–protein interactions, would be useful for understanding such complex phenomena.

High affinity molecules, such as nucleic acid ligands, can modulate the transcriptional activity of transcription factors. Reiterated in vitro selection procedures

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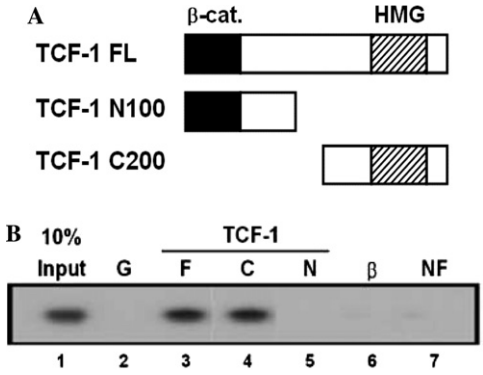

Fig. 1. Binding of RNA aptamer #10 to the DNA binding domain of TCF-1 protein. (A) Recombinant GST TCF-1 proteins used in this study. (B) Protein specificity of RNA aptamer #10. GST pull-down analysis was performed with labeled RNA aptamer #10 (100 pM) and diverse GST fusion proteins (500 nM). G, GST protein; F, full-length TCF-1 protein; C, TCF-1 C200; N, TCF-1 N100; β, β-catenin; and NF, NFAT protein.

are able to select specific RNA molecules from random RNA library, and nucleic acids selected by this procedure are generally referred to as 'aptamers' [26,27]. Because of the large size of RNA libraries (10^{14}–10^{15} molecules) and the ease of generating RNA molecules by in vitro enzymatic reactions, RNA libraries are superior to other biological or synthetic libraries for selecting high affinity aptamers [28], especially for those recognizing nucleic acid binding proteins such as TCF-1 transcription factor.

We previously reported the in vitro selection of RNA aptamers that bind to TCF-1, without giving further details of their function [29]. Here we have extended our study and shown that one of the selected RNA aptamers (RNA aptamer #10) binds to the TCF-1 protein containing HMG domain, thereby interfering with its binding to DNA. Further refinement of this aptamer could provide a way for cancer cell gene therapy.

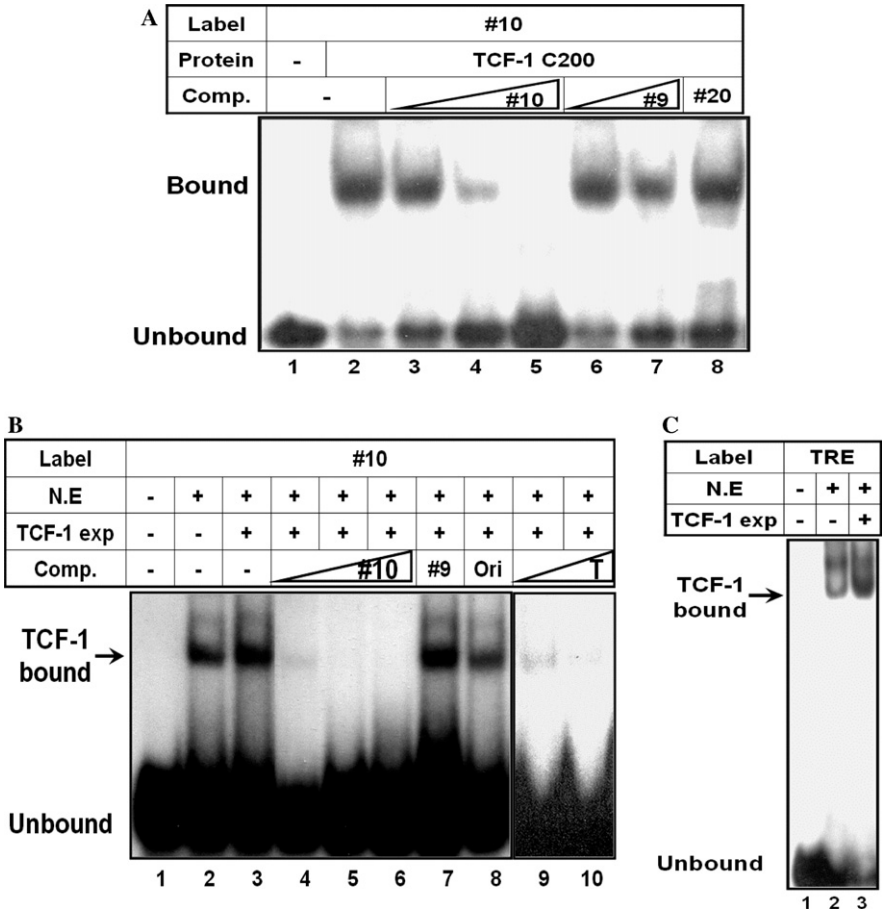

Fig. 2. Specific binding of RNA aptamer #10 to TCF-1 protein. (A) RNA-EMSAs were performed with labeled RNA aptamer #10 (500 pM) and TCF-1 C200 protein (500 nM) on its own, and in the presence of excess unlabeled RNA aptamer #10 (5, 50, and 500 nM), RNA #9 (50 and 500 nM), and RNA #20 (500 nM) as competitors. (B) Specific binding of RNA aptamer #10 to the cellular TCF-1 protein. RNA-EMSA was performed with labeled RNA aptamer #10 (100 pM) and 293T nuclear extract (N.E, 7 μg) in the presence of competitors. Lane 1, no extract; lane 2, vector transfected cells; lane 3, TCF-1 cDNA transfected cells; and lanes 4–8, with non-labeled RNA competitors, RNA aptamer #10 (20, 200, and 500 nM), RNA #9 (500 nM), and original RNA (Ori, 500 nM). The identity of the bound band is confirmed by the competition with the TCF-1 binding oligonucleotide, TRE (T, 100 nM and 500 nM, lanes 9–10). (C) Confirmation of the identity of the bound protein. EMSAs were performed with the TCF-1 binding oligonucleotide, TRE (20 nM), and nuclear extract (N.E) from 293T cells. Lane 1, no extract; lane 2, vector transfected cells; and lane 3, TCF-1 cDNA transfected cells. The TCF-1 bound band is indicated.