Molecular Therapy
Nucleic Acids
Commentary

Lockpicking FGFR1 with aptamer-based technology

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Targeting undruggable proteins and achieving high selectivity remain critical challenges in molecular therapeutics, as the lack of well-defined binding pockets, along with off-target effects leading to toxicity and reduced efficacy, often hampers drug development. The study by Zlinska et al.[1] leverages aptamer technology to develop new molecular and therapeutic tools for fibroblast growth factor receptor-1 (FGFR1) modulation. It describes a novel DNA G-quadruplex aptamer (called VZ23), which specifically binds to FGFR1 without affecting other FGFR variants, effectively inhibiting FGFR1 signaling and its cellular responses (Figure 1). This finding offers new potential solutions for treating several pathologies where FGFR1 is relevant, including cancer, skeletal and developmental disorders, and metabolic syndromes.

Current FGFR-targeted therapies rely on small-molecule tyrosine kinase inhibitors (TKIs), including erdafitinib (US Food and Drug Administration [FDA] approved for FGFR2/3-mutated bladder cancer), pemigatinib, and futibatinib (FDA approved for cholangiocarcinomas with FGFR2 alterations). However, these inhibitors lack specificity, as they generally target all FGFRs and other receptor tyrosine kinases to a certain degree, leading to off-target effects and toxicity that limit their clinical application. With the growing focus on reducing toxicities in targeted therapies, aptamers are emerging as an attractive class of targeting ligands, offering a promising solution to enhance precision in therapeutic design. Oligonucleotide aptamers are single-stranded DNA or RNA molecules, typically 20–80 nt long, that bind their targets with high affinity (in the nanomolar-to-picomolar range) and specificity due to their complex three-dimensional structures. Aptamers can be selected against a wide range of targets using the systematic evolution of ligands by exponential enrichment (SELEX), an iterative process based on combinatorial chemistry.[2] Aptamers, often referred to as "chemical antibodies", have diverse applications similar to traditional antibodies, with some differences: they are small, less antigenic *in vivo* (as they are not cell-derived proteins), have a limited half-life and circulation time, and can be produced on a large scale through *in vitro* synthesis. Additionally, their effects can be reversed using a universal antidote or a complementary oligonucleotide.[3] In the study by Zlinska et al.,[1] VZ23 has shown promising efficacy and, as a first-generation FGFR1 aptamer, it has the potential for further optimization. Its ability to inhibit FGFR1 signaling depends on its capacity to adopt an antiparallel G-quadruplex structure, which confers high thermodynamic stability to the aptamer. This discovery paves the way for a better understanding of the structure of VZ23 to inform the development of more stable and active derivatives. It also provides insights into how VZ23 binding blocks FGFR1's function precluding its intracellular signaling.

In this study, a commercial library of 10^{15} 76-nt DNA oligonucleotides with a 40-nt randomized region was screened using the extracellular domain (Arg22-Glu285) of human FGFR1c fused to the Fc domain of immunoglobulin G1 (IgG1) and immobilized on protein G magnetic beads. After 6 rounds of SELEX, 10 aptamers (A–J) were identified, but only aptamer I, designated VZ23, was further characterized due to its ability to completely inhibit ERK activity, a key marker of FGFR1c downstream signaling following FGF1 induction. Biolayer interferometry studies showed that VZ23 binds to FGFR1 variants with K_Ds of 55 nM (FGFR1b) and 162 nM (FGFR1c), but not to other FGFR variants (FGFR2b, FGFR2c, FGFR3b, FGFR3c, FGFR4). Of note, the K_Ds of FDA-approved erdafitinib are within 0.25 and 2.2 nM for all FGFR receptors, although they also present binding affinities within the same range to other kinases such as RET, CSF1R, PDGFRA, and VEGFR2.[4] However, VZ23 selectively inhibited its intended target without interfering with other receptors such as the insulin receptor, stem cell growth factor receptor, hepatocyte growth factor receptor, or epidermal growth factor receptor.

To investigate the structure of VZ23, nuclear magnetic resonance (NMR) spectroscopy and circular dichroism were used. The ^{1}H one-dimensional (1D) NMR spectra of VZ23 suggested that it adopts a non-canonical DNA structure stabilized by Watson-Crick and Hoogsteen base pairs, which depends on the concentration of Mg^{2+}; importantly, the folding was not compromised in the presence of fetal bovine serum. Analysis of imino-aromatic connectivity in ^{1}H–^{1}H 2D nuclear Overhauser effect spectroscopy confirmed that VZ23 adopts an antiparallel G-quadruplex-like folding topology. Remarkably, Mg^{2+}-induced stabilization of VZ23 led to a significant increase in its binding affinity for FGFR1, reducing its K_D by 45% for FGFR1b and 60% for FGFR1c. This is expected, as cations play a crucial role in stabilizing G-quadruplex structures by intercalating into the central cavity formed by stacked guanine tetrads. It may be worth exploring the use of other cations, such as potassium (K^+), which is known to be the most effective in stabilizing G-quadruplex structures due to its optimal ionic radius. In living cells, where K^+ and Na^+ are the predominant free cations, G-quadruplexes preferentially bind these ions, particularly K^+.

FGFR1c can be activated by multiple ligands, including FGF1, FGF2, and FGF4; treatment

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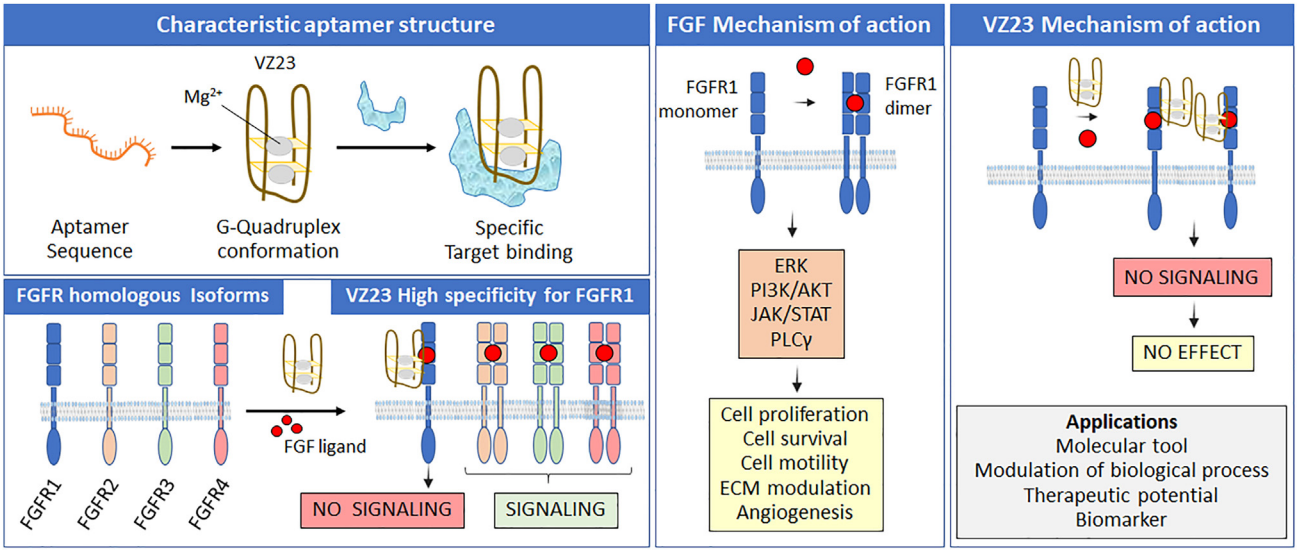

Figure 1. Schematic illustrations of the key features of VZ23, a novel DNA aptamer for selective FGFR1 modulation

FGFR1 can be activated by different FGF ligands, triggering diverse signaling cascades and biological processes. Aberrant activation of FGFRs has been associated with different pathologies, including cancer and skeletal and developmental disorders. VZ23 is a DNA aptamer generated through SELEX that adopts an antiparallel G-quadruplex structure. It exhibits high affinity and specificity for FGFR1 among all FGFR variants and other receptor tyrosine kinases. VZ23 blocks FGFR1 function, possibly by preventing receptor dimerization. These characteristics of VZ23, combined with the intrinsic advantages of aptamer-based tools and upon further optimization and preclinical validation, offer potential for a wide range of applications.

of rat chondrosarcoma (RCS) cells expressing FGFR1c with these ligands resulted in similar ERK activation levels, and inhibition by VZ23 was comparable across them, with half-maximal inhibitory concentration (IC_{50}) values of 0.57, 0.44, and 0.48 μM for FGF1, FGF2, and FGF4, respectively. VZ23 effectively blocked downstream FGFR1 signaling and inhibited FGFR1-mediated regulation of cellular senescence, proliferation, and extracellular matrix homeostasis. Furthermore, competitive binding experiments demonstrated that VZ23 does not interfere with FGF1 binding to FGFR1b or FGFR1c and can bind to preformed FGF1:FGFR1 complexes. Deleting the Ig1 domain (which has a regulatory function) did not abolish VZ23 binding to FGFR1. Altogether, these observations suggest that VZ23 binds to a distinct site from the FGF binding site (i.e., Ig2, Ig3) and does not involve Ig1. Mutational analysis of VZ23 (G2T, T14A, and G25T) revealed that different substitutions had varying effects on its stability and biological activity in RCS-FGFR1c cells. The precise mechanism by which VZ23 blocks the transmission of signals in FGFR1 remains unclear. While the authors suggest that VZ23 prevents dimer formation, the study does not explicitly confirm this. Further structural studies, such as docking experiments or cryoelectron microscopy, would be valuable to clarify the binding interactions, identify critical binding sites, and determine how VZ23 disrupts FGFR1 function. Finally, VZ23 was compared with zoligratinib, an FGFR-TKI tested in clinical trials for FGFR-related cancers. While zoligratinib inhibited FGF-induced ERK activation in RCS cells expressing only endogenous FGFR1c, FGFR2c, FGFR3c, and FGFR4, VZ23 selectively inhibited FGFR1c, showcasing its key advantage—high specificity. Although zoligratinib exhibited a lower IC_{50} for the inhibition of FGF1-FGFR1c signaling in RCS cells (0.13 μM) compared to VZ23 (0.57 μM), it is important to consider that VZ23 is a first-generation aptamer that has yet to be optimized. Of note, zoligratinib development was discontinued due to limited efficacy and safety concerns in clinical trials despite promising preclinical data. These important caveats may, at least in principle, be addressed with the further development of VZ23. Nonetheless, aptamer-based therapeutics still face some challenges, including limited stability *in vivo* due to nuclease degradation and difficulties in translocating across the cell membrane into the cytosol to target intracellular molecules. Delivery remains a hurdle, especially for crossing biological barriers like the blood-brain barrier, while modifications to enhance stability or pharmacokinetics can increase production complexity and cost. Additionally, scalability issues and limited clinical data with only two aptamers approved by the FDA further impact their widespread adoption compared to antibodies or small-molecule drugs. Advances in oligonucleotide chemical synthesis and the incorporation of novel chemical modifications could drive the development of this technology for broader clinical applications in the future.

In summary, this study introduces an innovative molecular tool for FGFR1 modulation, which, upon further optimization and preclinical validation, may represent a significant advancement in the field. The findings suggest that DNA aptamers could be an effective alternative to TKIs for treating FGFR1-related dysfunctions, implicated in

over 10 types of cancer and conditions such as human craniosynostoses. VZ23 may have broader potential applications. Due to the low cost of DNA aptamer synthesis, long shelf life, and potential for covalent chemical modifications (e.g., fluorophores), VZ23 may serve as a viable alternative to antibodies for cell population isolation and bioimaging approaches, or as a foundation for FGFR1-specific affinity matrices.[3] In the future, this aptamer after further optimization could be chemically synthesized for large-scale production and, if internalized, conjugated to other oligonucleotide cargoes, such as small interfering RNA,[5] or combined with other aptamers to form dual-specificity chimeras, expanding their applications in protein regulation and intracellular targeting.[6] Overall, this study represents a clear example for alternative nucleotide-based therapeutics, illustrating how aptamers selected via SELEX can exhibit exquisite selectivity for specific protein isoforms, which is sometimes difficult to achieve with small molecules or even antibodies.

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AUTHOR CONTRIBUTIONS

F.N., F.P., and F.C. wrote the manuscript. F.N. and F.C. created the artwork.

DECLARATION OF INTERESTS

The authors declare no competing interests. F.P. is associate editor of this journal.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGFP from OpenAI to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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