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PNA: A Snapshot on their Applications in Leukemia Diagnosis and Research

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Abstract

PNAs are oligomers with a backbone composed of N-(2- aminoethyl) glycine units. They are chemically stable and resistant to hydrolytic cleavage and can hybridize to complementary DNA and RNA. PNA also show greater specificity and are stable over a wide range of pH and temperature. The unique characteristics of PNAs have allowed the development of a lot of different biological assays. In our opinion paper, we summarized the PNA applications focusing on the diagnosis of leukemia.

Keywords : PNA; FISH; PCR clamping; ddPCR; Leukemia diagnosis

Abbreviations: PNAs: Peptide Nucleic Acids; FISH: Fluorescence *In Situ* Hybridization; Tm: Melting Temperature; RT-PCR: Real Time PCR; ddPCR: Droplet Digital PCR

Introduction

The Peptide Nucleic Acids (PNAs) are synthetic organic polymer similar to DNA and RNA, with differences in structure composition [1,2]. While DNA and RNA have a skeleton formed by the sugars deoxyribose and ribose respectively, PNA is composed of repeating units of N-(2-aminoethyl)-glycine, joined by peptide bonds. The purine and pyrimidine bases are linked to the skeleton via methylene carbonyls linkages [3]. PNA does not contain phosphate groups, so the PNA/DNA is stronger than DNA/DNA duplex, due to reduced electrostatic repulsion. The high affinity of PNA to bind DNA and RNA allows the use of very short PNA oligomers (13-20bp). PNA oligomers also show greater specificity and are stable over a wide pH range. Furthermore, their electrically neutral nature may facilitate passage through the cell membrane, increasing the therapeutic value [3]. PNA shows preferably a helical conformation called "P-form", different from any other stranded nucleic acids [4]. The structural characteristics of the PNA make it suitable for further chemical modifications, such as the replacement of units of glycine with any other amino acid.

PNAs were originally created for DNA binding, in order to study the mechanism of double helix invasion. Subsequently, they have been applied in microbiology, virology and pharmacology and recently in genetics and cytogenetics. PNAs have important chemical-physical properties exploited for the development of biomolecular tools, such as molecular probes, biosensors and antisense agents [1].

In recent years, PNAs have emerged for many applications in cancer diagnosis and experimental therapy due to their peculiar characteristics. In this opinion, we summarized the recent progresses in leukemia diagnosis using PNA biotechnology.

PNA-FISH

The use of PNA probes in Fluorescence In Situ Hybridization (FISH) analysis is one of the most interesting applications [5]. PNAs are compatible with a wide range of molecules reporters and fluorochromes [3]. The physical properties of PNAs endow them with specific advantages over standard oligonucleotides probes: low intensity of background signal and high stability [6]. Initially, the technique of the PNA-FISH has been used for the quantitative analysis of telomeres [5,7]. The discriminatory characteristic of PNAs can also increase the identification of chromosomal variations and analysis of polymorphisms, through the synthesis of allele-specific PNA [8]. Furthermore, these unique features have been exploited to design assays able to highlight single base mutations at single cell level (e.g. Bcr-Abl T315I, JAK2 V617F), undetectable with traditional FISH test [9-11].

PNA mediated PCR clamping

Another application of PNA probe concerns the PCR. PNA/DNA chimeras cannot be recognized by DNA polymerase, suppressing the PCR reaction. This strategy, called PNA-mediated PCR clamping, uses PNA probes to inhibit the amplification of

a specific target sequence. The direct competition between PNA probe and conventional primer allows the amplification of interesting sequence. The lower charge repulsion between PNA and DNA favours the melting temperature (Tm) to remain independent from the concentration of salts [3]. Therefore, this procedure can be used to identify genetic variants [12], single base mutation [11] or mutations involving wider genomic regions (e.g. deletions and insertions) [13]. Although DNA sequencing is the most common diagnostic method and allows to analyze the entire sequence of interest, this PNA based assay resulted to be more sensitive, specific and cheaper.

PNA mediated real time PCR clamping

Method based on PNA Real Time PCR (RT-PCR) Clamping allows the quantification of specific mutations or variants at known positions. The PNA probe is designed to bind the wild type [14] sequence of the gene of interest, inhibiting its amplification. PNA can compete with either a TaqMan probe [15,16] or with a DNA primer when using Sybr Green as a DNA intercalates [17]. This direct competition allows the amplification and quantification of interesting sequence (e.g. DNMT3A R882H). Considering the high cost and the relatively low sensitivity of the DNA sequencing method, the PNA RT-PCR assay results faster and more sensitive in detecting and monitoring low level mutations in clinical samples.

PNA mediated droplet digital PCR

The Droplet digital PCR (ddPCR) is one of the most sensitive method for measuring target nucleic acid sequence quantity, it is useful for determining somatic mutation rates using TaqMan probes. By combination of PCR with PNA and ddPCR (PNA–ddPCR), it may be possible to successfully detect low prevalent mutant alleles more sensitively than with ddPCR alone [18]. Furthermore, the use of PNAs in ddPCR allows to improve also the specificity of the usual ddPCR [19].

PNA target in vitro and in vivo

The use of normal and thioate antisense oligonucleotides in vitro and in vivo displayed a number of limits: nuclease degradation, insufficient target affinity and non-specific effects. By contrast, the PNAs stability in biological fluids and their high affinity hybridization characteristics suggest that PNAs could be used as antisense agents in vitro and in vivo. If PNAs were conjugated with Nuclear Localisation Signal peptide (NLS), they can cross cell membrane and interfere with the stability of target mRNAs and proteins translation [20]. Similar results were obtained with in vivo injection in rat brain [21]. Further study showed that PNAs are also able to cross the blood-brain barrier [22].

These findings suggest that antisense PNAs could be considered a new strategy for targeting of cell-specific anti-gene therapy in cancer.

Conclusion

PNAs provide a novel class of organic polymer with wide biological potential as tools for diagnostic applications and therapeutic compounds. Their versatility, stability and easily to use, features widely discussed above, make them an excellent alternative to the most common methods, such as FISH and DNA sequencing. Their heir peculiar characteristics allow their use also in the most advanced techniques (ddPCR), improving in sensitivity and specificity. Furthermore, their potential use in vivo makes them excellent candidates for gene therapies, increasingly attractive in translational research.

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