Designing probe from E6 genome region of human Papillomavirus 16 for sensing applications

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ABSTRACT

Human Papillomavirus (HPV) is a standout amongst the most commonly reported over 100 types, among them genotypes 16, 18, 31 and 45 are the high-risk HPV. Herein, we designed the oligonucleotide probe for the detection of predominant HPV type 16 for the sensing applications. Conserved amino acid sequences within E6 region of the open reading frame in the HPV genome was used as the basis to design oligonucleotide probe to detect cervical cancer. Analyses of E6 amino acid sequences from the high-risk HPV were done to check the percentage of similarity and consensus regions that cause different cancers, including cervical cancer. Basic local alignment search tools (BLAST) have given extra statistical parameters, for example, desire values (E-values) and score bits. The probe, ‘GGG GTC GGT GGA CCG GTC GAT GTA’ was designed with 66.7% GC content. This oligonucleotide probe is designed with the length of 24 mer, GC percent is between 40 and 70, and the melting point (Tm) is above 50 °C. The probe needed an acceptable length between 22 and 31 mer. The choice of region is identified here can be used as a probe, has implications for HPV detection techniques in biosensor especially for clinical determination of cervical cancer.

1. Introduction

Over the last decade, amazing progress has been made to understand the pathogenesis of Human Papillomavirus (HPV). Examining the natural history and the study of disease transmission of HPV is complicated, because there are more than 100 strains of HPVs found [1–4]. Since all HPV sorts are immovably related, tests can be intended to target the conserved region of the genome or areas where sequences can best be used to isolate assorted HPV strains [5]. High-risk types are those like the sorts most of the time, were found in anogenital malignancies; by and large generally the low-risk types are those like the types related in condylomata [6–8]. There are several approaches to distinguish different types of HPV strains in clinical specimens. One of the basic ways is by utilizing primers coordinated to the generally conserved regions of HPV genome and after that, the consensus amplicon is recognized by the proper strategy, for example, dot blot hybridization with particular probe(s), restriction fragment length polymorphism examination, or sequencing [9–15].

Generally, cervical cancer is created by the high-risk types of HPV [16–18] and the genome association of HPV strain 16, is one of the subtypes with a specific genome is known to cause cervical cancer predominantly (Fig. 1). This HPV genome includes an upstream regulatory region (URR), or long control region (LCR), has early genes (E1, E2, E6, and E7), and late genes (L1 and L2). It ought to be noted, about overlapping adjacent regions: e.g., E6, which is more prominent than E7, covers a huge segment of E7 [19–23]. Integration is essential, goes before cancer growth development, and could be incorporated into the malignant change [24]. In cervical carcinoma cell lines, the viral deoxyribonucleic acid (DNA) is typically incorporated into the human cell genome [25–27]. Previously, integration form had been found as much as 44%, episomal had 44% and both forms had 11% for cervical carcinomas containing HPV 16 [16,28,29]. A technique including real-time PCR has been utilized to screen for mixing status by determination of the E2/E6 proportion and might be appropriate for checking disease progression. Combination includes breakage of the HPV DNA and loss of parts in genome. Early perceptions of cervical carcinoma cell lines uncovered erasure of 2–3 kb that incorporated the E2 to L2 area [30–32]. The most crucial part is E6–E7 transcripts could be recognized in the cells. Open reading frame (ORFs) of E1, E2, E4, and E5
were hindered by flanking the host cell DNA, indicating as sites of combination [33]. HPV mRNAs present hybridized probes for the whole E6 and E7 ORF and a minor part of the E1 ORF, implying that these were the main portions of the HPV genome present [34,35]. Investigation of six cervical carcinoma tests demonstrated that the E6/E7 region was observed to be held on joining, yet the E2 was lost. Viral genome integrated into human chromosomal DNA of cancer cells generally erases E2 ORF, which results in the loss of expression of the E2 gene, however, is connected with the continuation of high expression of E6 and E7 [36–42]. Whatever the rest of the genome may have a part in transmission of the infection to another host cell, yet it is the proteins encoded by E6 and E7 that cause the malignancy. Joining of HPV DNA happens early in cancer development and is vital as an enactment mechanism for movement from the precancer to cancer [43–46]. E6 and E7 regions demonstrate the best contrasts in nucleotide sequence between various HPV types and is constantly held by cells that are contaminated compared to other regions in HPV 16 [47–49].

DNA has a role as biomarker of HPV. Investigation of potential biomarker helps to identify a new pathway involved in the HPV pathogenesis to prevent cervical cancer. DNA probes are stretches of single-stranded used to detect the presence of complementary nucleic acid sequences; in other word, is a target sequence by hybridization process. HPV type-specific probes provide a rapid and cost-effective method to simultaneously detect HPV genotypes. Suitable DNA probe synthesize based on E6 area of the different types of HPV able to answer whether a patient is infected with a high-risk HPV, can be integrated and promptly fused into the sensing purposes [50,51]. The use of E6 DNA probes make the detection is considered to be applicable for early discovery of cervical cancer and expenses will be lower. The general principle of DNA biosensor involves the immobilization of a DNA or DNA-analog probe onto the electrode surface. The importance of DNA probe in the sensing application with biosensor has been shown to give a higher sensitivity and specificity, fast hybridization kinetic strength rather than ionic strength and requires only a shorter probe length [52]. Fig. 2 showed a research overview in the development of highly sensitive and selective nanoparticles based biosensor for early detection of cervical cancer.

To analyse the region of interest, BLAST programs are widely used as tools for looking protein and DNA databases for group-
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