Ultrasensitive Detection of Mutations in the Androgen Receptor Gene by ICE COLD-PCR

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ABSTRACT

The Androgen Receptor plays an important role in prostate cancer, androgen-dependent (AD) and androgen-independent (AI) disease progression. Anti-androgen therapies are the hallmark of prostate cancer treatment, however spontaneous AR mutations are often detected in hormone-refractory, androgen-ablated and metastatic tumors.

ICE COLD-PCR (Improved & Complete Enrichment CO-amplification at Lower Denaturation temperature) is a technology that preferentially enriches mutant DNA sequences in an excess of wild-type DNA through selective amplification of the mutant DNA population using an oligonucleotide complementary to wild-type sequence (RS-oligo). This RS-oligo prevents PCR amplification of wild-type sequences while allowing amplification of DNA containing any mutation covered in the RS-oligo region. We have developed an ICE COLD-PCR assay for the enrichment of mutations in the Androgen Receptor including the AR H874R, H874Y and T877A mutations. The mutant-enriched DNA was then analyzed by standard Sanger DNA sequencing. The analytical sensitivity for the ICE COLD-PCR assay was 1 in 10,000 (0.01%) for all the mutations, compared to 20% limit of detection by standard Sanger sequencing.

The ICE COLD-PCR assay developed here provides a means to detect low level mutations in the Androgen Receptor and thus will provide a valuable methodology for the early diagnosis, treatment guidance, outcome prediction and relapse monitoring of prostate cancer.

INTRODUCTION

Prostate cancer is one of the most common types of cancer in men (for reviews, see 1-5). Normal prostate and tumor growth is almost always dependent on the androgen receptor pathway (Fig.1) and anti-androgen therapies are the hallmark of prostate cancer treatment. Androgen ablation often leads to initial favorable response. However, patients invariably relapse with aggressive castration-resistant or hormone-refractory prostate cancer (CRPC). Several mechanisms for CRCP progression are involved with AR (Fig. 2). The functional significance of AR mutations in PCa is illustrated in an androgen dependent cell line LNCaP that harbors a T877A mutation. With the mutation, the growth of LNCaP is stimulated in vitro not only by androgens but also by nonandrogenic steroids. Overall, AR mutations are rarely found in untreated localized PCa but are detected at high frequencies in hormone-refractory, androgen-ablated and metastatic tumors. There have been differences in the reported incidences of AR mutations, which might be attributed to variability in AR signaling and the analytical sensitivities for AR mutation detection. For example, standard Sanger sequencing has a typical LOD of only 15~20%. Also, most biopsies are performed at the time of diagnosis or at surgery, which represent PCa many years ahead of the actual development of CRPC.

Ice COLD-PCR is a new technology that can selectively amplify mutant DNA templates (6). The amplification of normal wild type DNA is blocked by the perfectly matched RS oligo at a critical temperature Tc. However, the RS oligo does not anneal well to the mutant DNA template with a mismatch and the PCR amplification proceeds unimpeded (Fig. 3). We have developed an ICE COLD-PCR assay for the detection of Androgen Receptor mutations including H874R, H874Y and T877A. The limit of detection was 0.01% (1 in 10,000) for all the mutations, compared to 20% by standard Sanger sequencing. This extremely sensitive ICE COLD-PCR assay will serve a valuable tool for early prostate cancer detection/diagnosis, treatment guidance, outcome prediction, and relapse monitoring. It allows mutation detection on less invasive or more convenient samples sources such as plasma or CTC, where wild type DNA molecules are in large excess. The assay also offers an opportunity for much needed reassessment of the relationship between AR mutations and prostate cancer development.

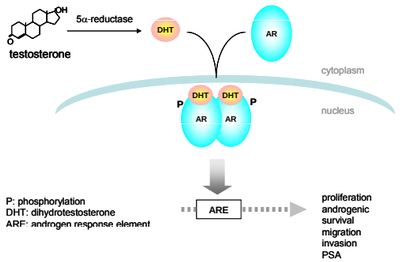

Fig. 1. Canonical AR pathway for normal prostate and cancer.

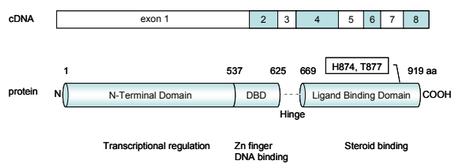

Fig. 2. AR gene exon structure and protein domains (top). Functional roles and AR mutations in prostate cancer development (bottom).

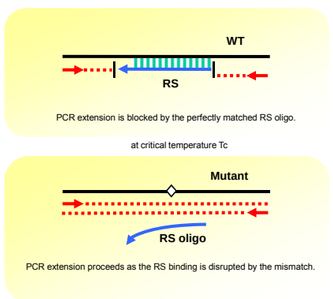

Fig. 3. Mutant enrichment by ICE COLD-PCR at a critical temperature Tc.

RESULTS

The mutations T877A (Fig 4.), H874R (Fig. 5) and H874Y (Fig. 6) were detected by ICE COLD-PCR at extremely high sensitivity at 0.01% (1 mutant in 10,000 wild type DNA molecules), compared to 10~20% by standard PCR. The LOD is equivalent to 1000~2000 fold enrichment for the mutants. This level of sensitivity has reached the theoretical lowest mutation level of 0.013% in a typical input genomic DNA amount of 25 ng (1 mutant in total of 7575 haploid genomes).

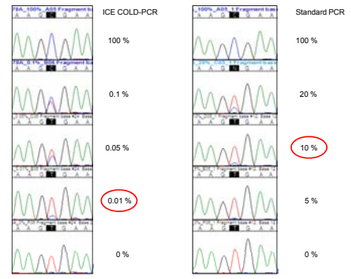

Fig. 4. The T877A (T>C) mutation detection limits by ICE COLD-PCR at 0.01% vs. standard sequencing at 10% (circled).

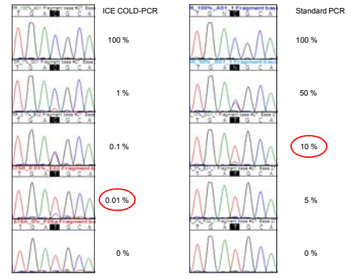

Fig. 5. The H874R (T>C) mutation detection limits by ICE COLD-PCR at 0.01% vs. standard sequencing at 10% (circled).

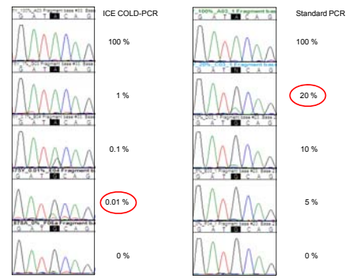

Fig. 6. The H874Y (G>A) mutation detection limits by ICE COLD-PCR at 0.01% vs. standard sequencing at 20% (circled).

MATERIALS AND METHODS

Locked Nucleic Acid (LNA) modified RS oligos were synthesized by Exiqon. Unmodified PCR primers were synthesized by Integrated DNA Technologies. The wild type genomic sequence of AR exon 8 was cloned into pNEB206A vector. The H874R, H874Y and T877A mutations were introduced into the wild type plasmid by site directed mutagenesis. Serial dilutions of the mutant DNA in the wild type DNA at the same concentration were prepared for the LOD studies.

The test DNA was initially pre-amplified by Phusion Polymerase (New England Biolabs). The pre-amplified material was used as the input DNA for ICE COLD-PCR. The ICE COLD-PCR reaction was optimized for primer and RS oligo concentrations using T-TAQ Polymerase (Transgenomic, Inc.). The reactions were carried out in a thermal cycler with precise thermal control. Post ICE COLD-PCR products were subjected to standard BigDye v3.1 sequencing. All sequencing reactions were analyzed on an ABI 3730 XL DNA Sequencer.

CONCLUSIONS

- ICE COLD-PCR and sequencing detected AR mutations H874R, H874Y, and T877A at a very high sensitivity. The LOD for all the mutations was 0.01%, compared to standard PCR/sequencing at 10~20%.
- For a typical 25 ng genomic DNA input, the assay has reached the maximum theoretical sensitivity of 1 mutant in 7550 haploid genomes (0.013%).
- Post ICE COLD-PCR sequencing uses standard sequencing reaction/capillary sequencing instrument, and offers familiar sequencing trace results, compared to other mutation detection methods such as HRM, qPCR, and heteroduplex analysis.
- This powerful assay may find its uses in prostate cancer research and diagnostic applications.

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