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Letters to the Editor

Correspondence re: J. B. de Kok *et al.*, DD3, A Very Sensitive and Specific Marker to Detect Prostate Tumors. Cancer Res., *62:* 2695–2698, 2002.

Letter

We read with great interest the article published in Cancer Research by de Kok *et al.* (1), who underlined the role of DD3 gene as a sensitive and specific marker of prostate cancer, thus suggesting its hypothetical role to identify circulating cancer cells in prostate cancer patients.

On the basis of these findings, to establish the clinical usefulness of this marker, we sought to investigate the DD3 gene expression in peripheral blood of patients with benign BPH and prostate cancer and in blood samples from healthy donors used as controls by reverse transcriptase-PCR assay.

On the basis of the sequence of DD3 gene presented in GenBank accession number AF103907, we choose primer sequences as follows: sense, located in exon 1: 5′-AGAAGCTGGCATCAGAAAAA-3′; antisense, located in exon 3: 5′-CTGGAAATGTGCAAAAACAT-3′; antisense, located in exon 4: 5′-TCCTGCCCATCCTTTAAGG-3′. Surprisingly, using the primer set located in exon 1 and exon 3, we detected DD3 product in all blood samples from normal controls (Fig. 1*A*). Additional analysis revealed the expression of DD3 exons 1 and 3 in MCF-7, M-14, HL-60, Hep-G2, and SKOV-3 cell lines, all derived from non prostatic tumors (Fig. 1*B*). To verify that what we were amplifying was really DD3 gene, all of the amplification products have been then digested with *Bgl*II restriction enzyme, which cuts once within the 233-bp PCR fragment. Sequence of the amplification products was the final confirm to this. From these data, we had to conclude that the region of DD3 located between exon 1 and exon 3 is not at all prostate specific. After an accurate revision of the articles from de Kok *et al.* (1) and Bussemakers *et al.* (2) previously, we deduced that what they actually amplified is the region between exon 1 and exon 4, and in Northern blot analysis, they used a probe located in exon 4. We thus thought that exon 4 may represent the really prostate-specific region of the DD3 gene. To confirm this hypothesis, we then analyzed RNAs from prostatic tissues, MCF-7, M-14, HL-60, Hep-G2, SKOV-3 and LNCaP cell lines, as well as neoplastic tissues from bladder, breast, and lymph nodes using as reverse primer the one located in exon 4.

We found the expected size DD3 band only in prostate cancer specimens, and in LNCaP cell line, derived from prostate cancer (Fig. 1*C*).

The above mentioned authors have already reported alternative splicing in the DD3 gene and deletion of exon 2 from most transcripts. Mechanisms of alternative splicing seem to be involved in the processing of mRNA precursors of DD3. Overall, these results suggest that DD3 mRNA may be expressed by alternative splicing, using tissue specific exon 4.

We conclude that the really prostate-specific region of DD3 gene is the splicing variant of exon 4; this observation has a crucial importance for the future use of DD3 gene as a specific marker of prostate cancer.

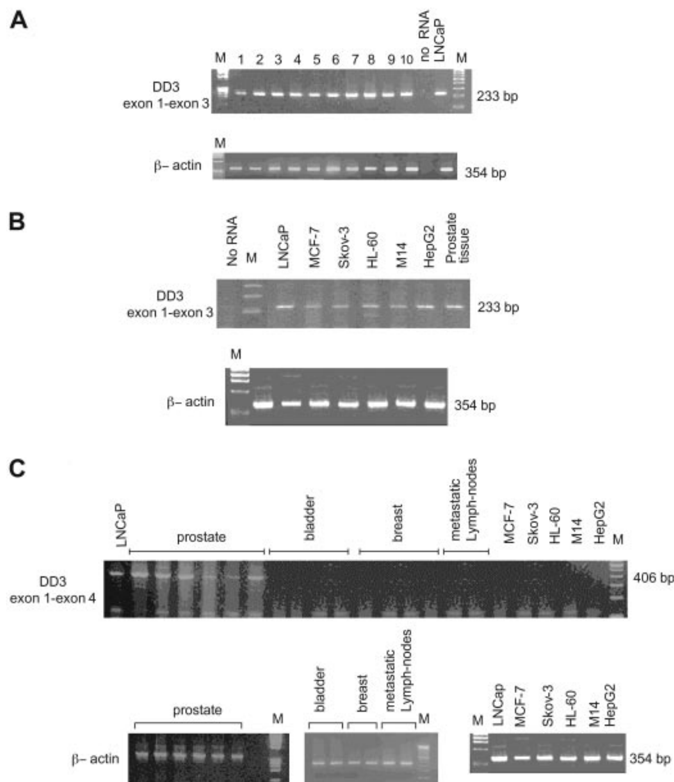

Fig. 1. Expression of DD3 gene evaluated by reverse transcriptase-PCR. *A*, expression of DD3 gene exon 1–exon 3 in blood samples from 10 healthy donors. No RNA: negative control; LNCaP positive control. *B*, expression of DD3 gene exon 1–exon 3 in human cell lines and in normal prostate tissue. *C*, expression of DD3 gene exon 1–exon 4 in normal prostate tissue, neoplastic, and nonneoplastic tissues (bladder, breast, and lymph nodes) and human cell lines. None of the nonprostatic tissue or nonprostatic cell lines investigated expressed the DD3 gene exon 1–exon 4. β-actin reverse transcriptase-PCR as internal control. M, molecular weight marker.

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