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LETTER TO THE EDITOR

# Correspondence Re: Ghosh A and Heston WDW. Tumor Target Prostate Specific Membrane Antigen (PSMA) and its Regulation in Prostate Cancer. J Cell Biochem 91:528–539, 2004

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## To the Editor

We read with great interest the article published in the *Journal of Cellular Biochemistry* by Ghosh and Heston [2004], reporting prostate specific membrane antigen (PSMA) as a unique membrane bound protein, which is overexpressed manifold in prostate cancer (CaP) as well as in the neovasculature of most solid tumors but not in the vasculature of normal tissues. PSMA is up-regulated in CaP, metastatic disease and hormone refractory CaPs, and its expression is modulated inversely by androgen levels. Owing to its intriguing though unexplained distribution, PSMA can serve as a detecting agent for metastatic foci of primary cancer. PSMA can also be used as a target of imaging agents to detect metastatic tumor sites, and also to detect prostate cells in circulation or lymphatics.

In contrast to Ghosh and Heston [2004] and in agreement with others [Grasso et al., 1998; Hara et al., 2002], we doubt the real usefulness of PSMA in detecting prostatic cells in peripheral blood (PB), in identifying patients at an increased risk of disseminated cancer and in defining the prognosis of disease. In a preliminary study, we amplified cDNA (250 ng) in PB from 42 patients with treated or untreated CaP and with benign prostatic hyperplasia (BPH) (controls) by PCR using the following primers: sense, located in exon 14: 5′AAAGTCCTTCCCCAGAGTTCA3′; antisense located in exon 17: 5′GAGTCTCTCACTGAACTTGGA3′ of PSMA [Israeli et al., 1993] using 30 cycles (94°C for 30 min, 57°C for 30 min, and 72°C for 60 s) and as control sense, located in exon 1: 5′AGCACAGAGCCTCGCCTTTG3′; antisense located in exon 4: 5′TGGCCATCTCTTGCTCGAAG3′ using 30 cycles (94°C for 30 min, 56°C for 30 min, and 72°C for 60 s) for human β-actin [Nakajima-Jijima et al., 1985]. We studied three clinical groups, 11 patients with CaP who underwent androgen ablation (AA) followed by retropubic radical prostatectomy (RP) (2 tumors were Gleason score sum 4, 7 Gleason sum 7, and 2 Gleason sum 8; 7 tumors were organ-confined diseases (T2 N0); and 4 were extra-prostatic diseases (T3b N0, N,1 and N2), follow-up after treatment ranged from 3 to 84 months (mean 27 months). At the time of venipuncture for PB, we distinguished two subgroups: patients who responded to the first 24 months of treatment without clinical progression; and AA resistant patients who received neoadjuvant androgen deprivation therapy because recurrent disease developed within 24 months after RP. The second subgroup comprised 11 patients with elevated serum PSA levels (more than 4.0 ng/ml), who had received a diagnosis of CaP

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**Fig. 1.** Analysis of gene expression levels of prostate specific membrane antigen (PSMA) by reverse transcription-polymerase chain reaction (RT-PCR) using β-actin as internal control, in whole blood from: (**A**) 11 patients after radical prostatectomy (RP) and androgen ablation (AA), RT-PCR β-actin shows a 740 base-pair band; negative control (**lane 12**); positive control (LNCaP cell line) (**lane 13**); MW (molecular weight). **B**: Eleven patients with prostate cancer (CaP) after biopsy and one patient with bladder cancer (**lane 12**) as control, negative control (**lane 13**); positive control (LNCaP cell line) (**lane 4**); MW.


**Fig. 2.** Analysis of gene expression levels of specific membrane antigen (PSMA) by RT-PCR using β-actin as internal control in: (**A**) whole blood from 18 of 20 patients with benign prostatic hyperplasia (BPH); (**B**) in frozen benign (**lanes 1**, **3**, and **6**) and neoplastic (**lanes 2**, **4**, and **5**) prostatic tissue; non prostatic tissue (adrenal gland, **lane 7**; heart, **lanes 8–10**; myometrium, **lane 11**; uterine cervix, **lane 12**) and human cell lines: SV48, **lane 13**; LS147D, **lane 14**; SCOV 3, **lane 15**; MCF7, **lane 16**; PC3, **lane 17**; and LNCaP, **lane 18**). Negative control no RNA (**lane 19**); MW.

based on TRUS-guided-prostate biopsy (1 tumor was Gleason sum 6 and 10 were Gleason sum 7). All 11 patients had organ-confined disease. In patients treated for CaP, PB samples were obtained 27–84 months (mean 55 months) after treatment ended; in patients who had received a diagnosis of CaP based on clinical and biochemical evidence of malignancy PB samples were obtained before biopsy and before treatment began. The control group comprised 20 men 50 years of age or older with no known malignancy, with a histologic diagnosis of BPH.

We found PSMA circulating cells more frequently in PB from treated than from untreated patients or controls (7/11 vs. 2/11 vs. 3/20). Reverse transcription-polymerase chain reaction (RT-PCR) analysis amplified PSMA positive cells in 7 (63.63%) of 11 treated patients with and without disease recurrence, in 2 (18.18%) of 11 untreated patients with CaP (Fig. 1), and in 3 (15%) of 20 patients with BPH diagnosed after biopsy (control group) (Fig. 2).

In agreement with Ghosh and Heston [2004], these data strongly argue that AA therapy increases PSMA expression. This increase might explain why in our PB samples RT-PCR detected PSMA-mRNA in a larger number of patients in our study than in others [Israeli et al., 1993]. RT-PCR detected PSMA-positive cells more frequently in treated patients with recurrent disease than in those without, and also in a higher percentage of patients with higher-grade Gleason scores than lower-grade scores. These data receive support from the finding of Ghosh and Heston [2004] that PSMA is up-regulated in CaP, metastatic disease, and hormone refractory cancer prostates and its expression is modulated inversely by androgen levels.

In our control tissues and cell lines, the 50% of neoplastic and non neoplastic prostatic tissue expressed PSMA, whereas non prostatic tissue did not. Only one non prostatic cell line (SKOW 3) and both LNCap and PC3 prostatic cell lines used as controls expressed PSMA-mRNA (Fig. 2). These findings are far from those of Ghosh and Heston [2004] reporting PSMA expression in CaP as well as in most solid tumors but not in normal tissues.

Yet despite reports that PSMA-PCR seems more sensitive than PSA-PCR as an indicator of disseminating prostate cells [Nakajima-Jijima et al., 1985], in our study it also yielded 15% false-positive results in BPH and also in a non prostatic (SKOW 3) cell line [Lanes et al., 2002]. These false-positive results probably arose because the primers we used amplify the splicing variant PSM′ [Grauer et al., 1998; Gala et al., 1998].

We, therefore, conclude that the *PSMA* gene lacks specificity for detecting prostate epithelial cells. Nevertheless the presence of PSMA-mRNA also in patients without CaP does not necessarily imply the presence of circulating neoplastic cells in PB. Circulating cancer cells are commonly found in PB from untreated patients with apparently localized cancers [Martìnez-Pineiro et al., 2003].

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