DIAGNOSTIC AND IMMUNO-THERAPEUTIC EVALUATION OF MONOCLONAL ANTIBODIES FOR THE EXTRACELLULAR DOMAIN OF PROSTATE-SPECIFIC MEMBRANE ANTIGEN

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ABSTRACT

This work reports the *in vitro* characterization and evaluation of three other commonly used mAbs (J415, J533, and J591) that bind the extracellular domain of Prostate-specific membrane antigen ($PSMA_{ext}$). Briefly, murine mAbs J415, J533, J591, and 7E11 were radiolabeled with ^{131}I and evaluated in competitive and saturation binding studies with substrates derived from LNCaP cells. J415 and J591 were conjugated to 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N*‴-tetraacetic acid labeled with ^{111}In. The uptake and cellular processing of these antibodies were evaluated in viable LNCaP cells. Competition assays revealed that J415 and J591 compete for binding to $PSMA_{ext}$ antigen. J533 bound to a region close to the J591 binding epitope, but J533 did not interfere with J415 binding to PSMA. 7E11 mAb did not inhibit the binding of J415, J533, or J591 (or *vice versa*) whereas 7E11 binds the intracellular domain of PSMA. Saturation binding studies demonstrated that J415 and J591 bound with a similar affinity (K_ds 1.76 and 1.83 nM), whereas J533 had a lower affinity (K_d, 18 nM). In parallel studies performed with viable LNCaP cells, J415, J533, and J591 bound to a similar number of PSMA sites (*i.e.,* 600,000–800,000 sites/cell), whereas 7E11 bound only to a subpopulation of the available PSMA sites (95,000 sites/cell). Up to five DOTA chelates could be bound to either J415 or J591 without compromising immunoreactivity. A comparison of the cellular uptake and metabolic processing of the ^{131}I- and ^{111}In-labeled antibodies showed a rapid elimination of ^{131}I from the cell and a high retention of ^{111}In. All four mAbs recognized and bound to similar numbers of PSMAs expressed by ruptured LNCaP cells (*i.e.*, the exposed intracellular and extracellular domains of PSMA). By comparison to J415 and J591, J533 had a lower binding affinity. Both J415 and J591 recognized and bound to the same high number of PSMAs expressed by intact LNCaP. By contrast, 7E11 bound to fewer sites expressed by intact LNCaP cells (*i.e.*, the exposed extracellular domain of PSMA). Both J415 and J591 are hence promising mAbs for the targeting of viable PSMA-expressing tissue with diagnostic and therapeutic metallic radionuclides.

KEYWORDS: PCa, prostate cancer; mAb, monoclonal antibody; PSMA, prostate-specific membrane antigen; DOTA, 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid; HPLC, high-performance liquid chromatography; DTPA, diethylenetriaminepentaacetic acid.