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Dissecting the Proteoform Landscape of Prostate-Specific Antigen: Intact, Bottom-Up, and Glycomic Perspectives

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Early detection of prostate cancer (PCa) using prostate-specific antigen (PSA) serum levels suffers from poor specificity and sensitivity, frequently causing unnecessary treatment or missed diagnoses. To overcome this, detailed characterization of PSA proteoforms, especially regarding glycosylation, is essential. Here, we employed an integrated analytical strategy using capillary electrophoresis coupled with mass spectrometry (CE-MS), progressively scaling from micro-level glycan details to macro-level intact protein analysis.

At the micro-level, released *N*-glycan profiling provided validation of glycosylation patterns identified by peptide-level (bottom-up) and intact analyses. The bottom-up approach offered detailed differentiation of glycopeptides, particularly distinguishing α2,3- from α2,6-linked sialic acid isomers through differences in electrophoretic mobility correlated with subtle pKa variations (relative pKa difference: 3.4×10^{-2}). This high-resolution separation uniquely revealed precise structural features, including the first identification of ketodeoxynononic acid (Kdn) on PSA glycans derived from seminal plasma and urine.

Moving to the macro-level, intact protein analysis delivered a global view of PSA proteoforms, capturing six proteolytic cleavage variants alongside diverse glycosylation states—including tri-, di-, mono-, and non-sialylated glycans—and, for in one of the patients urinary samples, uncovered a second glycosylation site resulting from genetic mutation.

Together, these complementary methodologies overcome individual analytical limitations, offering an extensive characterization of PSA proteoforms and their glycomic complexity. Future research will evaluate whether the proteoform diversity can be utilized to enhance discrimination between aggressive PCa, indolent PCa, and benign prostate hyperplasia. Additionally, further studies will investigate glycomic variations in plasma, preserve native PSA molecular complexes, and assess the clinical relevance of proteoform diversity in relation to disease severity and progression in larger patient cohorts.

User consent

yes

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