PREDICTION OF PROSTATE CANCER GLEASON GRADES BASED ON BLOOD/URINE LEVELS OF PROSTATE SPECIFIC ANTIGEN (PSA) DECORATED WITH HIGH MANNOSE N-GLYCAN

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PURPOSE

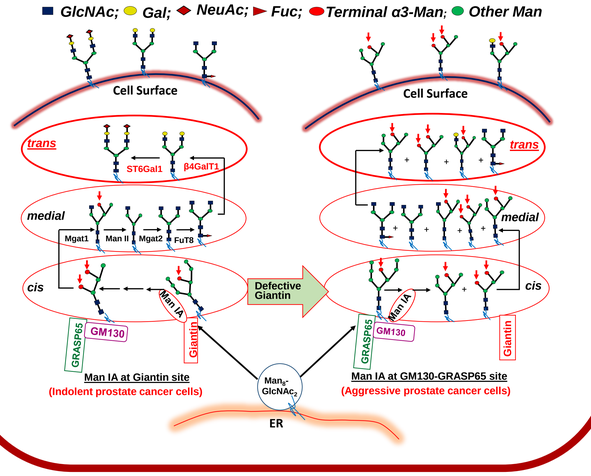
To develop a modified PSA assay for prediction of prostate cancer Gleason grade based on levels of PSA decorated with high mannose N-glycans in blood or urine

Background

(A) A non-invasive assay for prediction of prostate cancer Gleason grades is needed to aid the treatment decision: The inability of using serum PSA levels to distinguish advanced from indolent prostate cancer has resulted in over-treatment of clinically insignificant disease, thus causing unnecessary suffering of many patients (1,2). Therefore, there is a pressing need to develop a non-invasive assay that can predict prostate cancer Gleason grade, especially one that can distinguish Gleason scores ≤6 from Gleason scores ≥3+4 (3).

(B) Scientific basis of this project: Recently, Dr. Cheng's laboratory found that giantin, a Golgi matrix protein serving as a major targeting site for vesicles transporting glycosylation enzymes and their substrates from Endoplasmic Reticulum, lost its function as prostate cancer cells advanced from androgen-dependent to androgen-refractory stages. As a result, all but core 2 enzymes used GM130-GRASP65 site for Golgi targeting (4), causing alteration of mucin O-glycosylation (3) and N-glycosylation (5). Figure 1 illustrates how shifting of Golgi localization of α-mannosidase IA (Man IA), a key enzyme involved in trimming $Man_{6\text{-}8}GlcNAc_2$ down to $Man_5GlcNAc_2$ to enable synthesis of complex-type N-glycans, from giantin to GM130-GRASP65 site results in formation of high mannose (Man) N-glycans on *trans*-Golgi enzymes and cell surface glycoproteins (5). **Same alteration of N-glycan also occurs to prostate tumor-derived PSA, a secreted glycoprotein containing only one N-glycan chain, suggesting that levels of blood/urine PSA decorated with high Man N-glycan reflect prostate cancer Gleason grades.**

Fig. 1 Defective giantin in aggressive prostate cancer cells causes altered Golgi targeting of glycosylation enzymes, resulting in formation of α3Man-terminated N-glycans (|) in *trans*-Golgi enzymes, and cell surface and secreted glycoproteins



(C) Localization of Man 1A at GM130/GRASP65 site and expression of cell surface high Man N-glycans correlate with aggressive phenotype of prostate cancer cells: After extended culture of androgen-dependent prostate cancer cells (LNCaP P8), some become androgen refractory (6) (LNCaP-P121-Clone 1), resulting in a shift of Man 1A localization from giantin to GM130/GRASP65 (Fig. 2), appearance of high Man N-glycans at cell surface (Fig. 3), and an increase in *in vitro* migration rate (Table 1)

Fig. 2 Man IA co-localizes with Giantin in LNCaP-P8 and LNCaP-P121-C4 cells but with GM130/GRASP65 in LNCaP-P121-C1 and DU145 cells

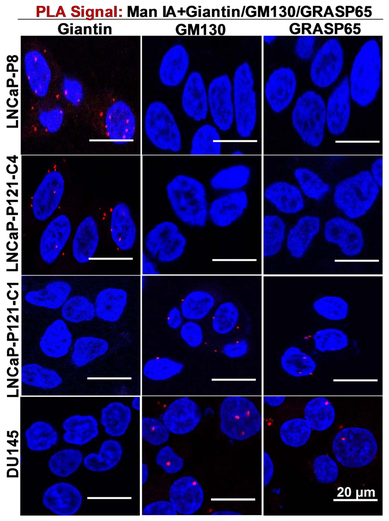


***Fig. 3 Galanthus nivalis* lectin (GNL) stain of cell surface N-glycans terminated with α3Mannose in LNCaP-P121-C1 and DU145 cells but not LNCaP-P121-C4 and LNCaP-P8 cells**

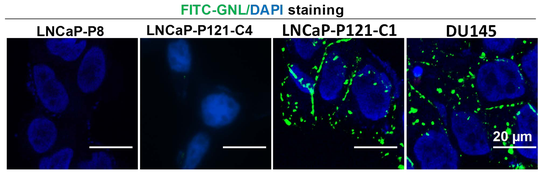
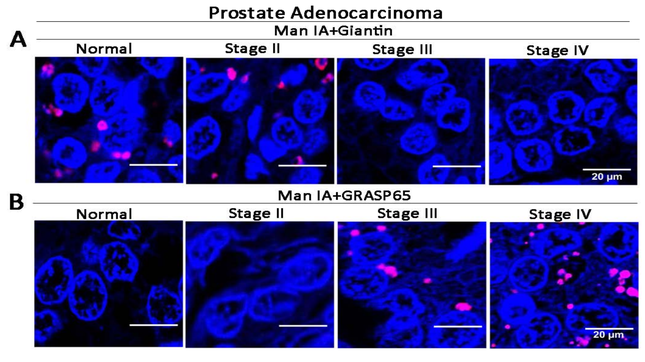


Table 1 Summary of Man IA localization, GNL stain, and scratch assay: LNCaP-P8 and LNCaP-P121-C4 cells vs. LNCaP-P121-C1 and DU146 cells

Cell Lines	Man IA Localization Site			GNL Staining	Scratch Assay Migration (μm/h)
	Giantin	GM130	GRASP65		
LNCaP-P8	+	–	–	–	3.8
LNCaP-P121-C4	+	–	–	–	5.0
LNCaP-P121-C1	–	+	+	+	10.9
DU145	–	+	+	+	9.1

(D) Man 1A is localized at Giantin site in normal prostate and stage II prostate tumors but at GM130-GRASP65 site in Stages III and IV prostate tumors: (Fig. 5)

Fig. 5 Proximity Ligation Assay (PLA) shows Man IA co-localizes with Giantin in normal prostate and stage II tumor and with GRASP65 in stages III & IV tumors



(E) High Man N-glycan was detected in PSA secreted from androgen-refractory prostate cancer cells (LNCaP P 45, DU145 and PC3) but not androgen-dependent prostate cancer cells (LNCaP P6)(Fig. 6) and also in serum/plasma of prostate cancer patient but not benign prostate hyperplasia patient (Fig. 7)

Fig. 6 Western blot of PSA and GNL in the conditioned media of LNCaP P6 & P45, DU145 and PC3 cells

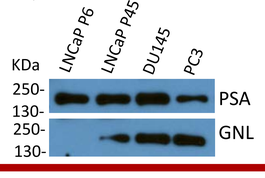
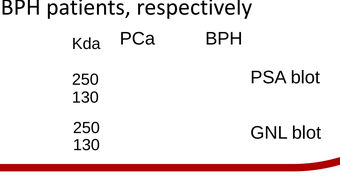


Fig. 7 PSA and GNL blotting of immunoprecipitated PSA from plasma and serum of prostate cancer (PCa) and BPH patients, respectively



AIMS

1. To show that PSA with high Man N-glycan is produced by aggressive prostate cancer cells
2. To validate that levels of PSA with high Man N-glycan in blood and urine correlate with Gleason ≤6, 3+4, 4+3, and ≥8 prostate cancer patients
3. To develop immuno and lectin combination Elisa assay of levels of PSA with high Man N-glycan in above mentioned specimens

APPROACH

1. Characterize *in vitro* migration and invasion property of prostate cancer cells with Golgi localization of Man IA at giantin or GM130-GRASP65 site and analyze N-glycan on secreted PSA.
2. Correlate, by western and lectin blotting, levels of PSA with high Man N-glycan in PSA isolated by immunoprecipitation from serum/urine of Gleason ≤6, 3+4, 4+3, and ≥8 patients.
3. Establish an Elisa assay for determination of levels of PSA with high Man N-glycan in urine and blood to predict Gleason grades.

NEXT STEPS/DELIVERABLES

After validate the concept that levels of PSA with high Man N-glycan in serum/urine can be used to predict prostate cancer Gleason grade, we will file a patent application and submit NIH RO1 or SBIR grant, DOD Idea award, or VA Merit.

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