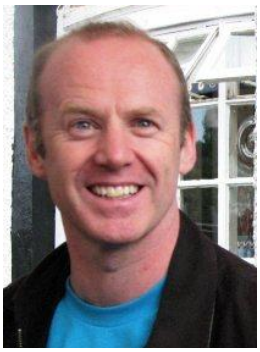
NANOTECHNOLOGY-BASED α_1-ADRENOCEPTOR/GPR55 BIOMARKER ASSAY: MOLECULAR PROGNOSTIC TEST FOR AGGRESSIVE PROSTATE CANCER ON THE HORIZON?



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The paramount clinical dilemma in prostate cancer (PCa) management is the search for reliable prognostic markers evaluable on biopsy specimens to enable minimization of intervention, incurring financial and morbidity-related costs. Although, in recent years, the application of next-generation sequencing to hundreds of prostate tumours has defined novel molecular sub-types, the unambiguous demonstration of tumour multifocality, clinical variability and transcriptomic diversity has confounded attempts to achieve diagnostic, prognostic and therapeutic breakthroughs. Currently, the majority of prognostic weight of PCa falls on Gleason grading and score and pre-treatment prostate specific antigen (PSA) levels [1], but in practice, even when combined with predictive nomograms, they do not provide sufficient information to accurately stratify tumours and guide clinical decision-making in patients. The unreliability of such traditional clinical and pathologic prognosticators, gaps in PCa armamentarium and high metastasis-associated deaths has prompted the search for prognostic tumour markers for metastatic behavior to allow discernment of patients who need and warrant radical therapy for aggressive disease from patients enrolled in 'active surveillance' group, dramatically reducing unnecessary complications, treatment and health-care costs.

Emerging experimental and clinical data has presented G-protein-coupled receptors (GPCRs) and their downstream-activated effectors a rich source of potential targets for drug delivery and ideal candidates for tumour imaging and biomarkers heralding malignancy/metastasis [2]. Signal transduction research investigating mechanisms of androgen-independent PCa cell proliferation has raised the idea that intracellular signaling mechanisms triggered through activation of α_1-adrenoceptors (α_1-ARs) and an 'atypical' cannabinoid receptor – G-Protein-Coupled Receptor 55 (GPR55), classical members of the GPCR family, mediate mitogenic effects in PCa and contribute to cell growth [3].

Mechanistic, translational and pharmacological studies that have enabled identification and characterization of structure, localization, expression profile and binding characteristics of GPCRs has provided a molecular platform for the development of novel high-affinity fluorescent ligands, resulting in more effective approaches for examining receptor distribution and degree of heterogeneity in living cells/tissues. Fluorescent BODIPY (derived from *bo*ron*dipy*rromethene) dyes have become 'newel Dorado's' for fluorescent labels and probes by virtue of their superior photochemical and photophysical properties [4,5,6]. Daly *et al.* (1998) have described the applicability of a high affinity (nM) pharmacological "antagonist" ligand termed QAPB (an abbreviation of quinazolinyl piperazine-BODIPY) related to the α_1-AR antagonist, Prazosin with an identical quinazolinyl piperazine group in quantitative non-radioactive assays to localize α_1-ARs and quantify receptor binding characteristics in live cells[7]. Similarly, the use of Tetramethylrhodamine (TMR) dyes that have gained attention as efficient lasing fluorophores due to its high fluorescence quantum yields, intense absorption and high lasing efficiency, has been reported in fluorescent based assay in the cannabinoid field. Studies have presented a newly commercialized fluorescent analogue of the CB1 receptor antagonist AM251, namely T1117, derivatized with a TMR (5-carboxytetramethylrhodamine; 5-TAMRA) moiety [8] as a novel fluorescent diarylpyrazole GPR55 ligand with little or no affinity to CB1 receptors.

However, typical BODIPY dyes have certain drawbacks such as hydrophobicity, relatively low wavelength absorption and emission maxima (λ_{max} around 500 nm) [9]. Methods applied to prepare BODIPY dye with red-shifted emission lead to a decreased solubility of the targeted fluorophore in aqueous solution or reduced fluorescence quantum yields [10] further limiting their full utilization for various biomedical and bioanalytical applications. Also, the TMR derivatives - TAMRA and the isothiocyanate derivative of TMR (TRITC) are quite hydrophobic when compared with their fluorescein counterparts conferring them the tendency to aggregate in aqueous solutions under conditions where the labeling density is sufficient to permit dye-dye interactions. A further consequence of these intermolecular interactions is fluorescence self-quenching which reduces the fluorescence output of the conjugate and increased complexity of the absorption spectrum of TMR-labeled proteins, usually splitting into two absorption peaks at about 520 and 550 nm making the actual degree of labeling difficult to determine [11].

The unique pharmacological, biochemical and physicochemical properties of nanomaterials have been exploited to design attractive nanoplatforms for the development of biotargeted biocompatible luminescent tracers and overcome the potential hurdles outlined. A supplementary advantage to encapsulation of dyes into nanoparticles (NPs) is (1) to enhance the detection limit by encapsulating a larger number of fluorophore molecules (2) to increase biocompatibility of the dye (3) to enhance brightness per particle over single molecule delivery (4) to increase sensibility to the buffer composition, preventing pH-mediated quenching or decomposition and (5) to produce monodisperse robust emitters from organic dye molecules and suitable nanospheres.

Pharmacological analysis of synergism or functional antagonism between members of the GPCR family has suggested a potential level of receptor complexity that could account for previously unexpected pharmacological diversities. Very recently, fluorescent ligand binding studies in human androgen-insensitive PC-3 and androgen-sensitive LNCaP prostatic carcinoma cell lines, using the fluorescent ligands - Syto 62 (nuclear stain), BODIPY FL-Prazosin (QAPB; fluorescent quinazoline α_1-AR ligand) and Tocriflour (T1117; fluorescent diarylpyrazole GPR55 ligand) has suggested the presence of subtype-rich cells with a degree of co-localization between α_1-ARs and GPR55 indicating a possibility for dimerisation or functional interaction and a new paradigm for functional synergism in which interactions may be either between cells or involve converging intracellular signaling processes [12]. Importantly, the study (for the first time) clearly demonstrated that α_1-AR co-expression results in markedly enhanced intracellular GPR55 expression in heterologous cells, particularly, to 'active' areas enriched with signaling molecules on the removal of α_1-antagonism after chronic administration of α_1-AR antagonist, Doxazosin. It is thus plausible that complex interaction between the two receptors supports tumorigenesis by promoting cell growth and drug resistance through (1) compensatory α_1-AR upregulation considered as an adaptive response to chronic administration of Doxazosin and (2) intriguing GPR55 upregulation suggestive of the off-target effects of α_1-AR antagonist in addition to the blockade of α_1-AR and demonstrating the significant impact of α_1-AR antagonists, particularly, Doxazosin, in PCa [12].

In the light of these findings, we proposed α_1-AR/GPR55 ratio as 'progression-associated prognosticator' in PCa which would aid in reliable forecasting of the prognostic or predictive status of the patient. Therefore, we believe it is possible to develop a novel bioassay for human prostate biopsies to predict the development of aggressive (GPR55-driven) PCa.

Emerging molecular imaging techniques have transformed "image-guided prognosis" into "molecular imaging-guided prognosis", improving the precision of prognosis and promoting effective therapy. Based on it, a tissue/cell assay employing NPs, with covalently encapsulated fluorescent ligands (to minimize any fluorophore leakage) - BODIPY FL-Prazosin (QAPB) and Tocriflour (T1117) can be designed and developed, as candidate clinical imaging modality for PCa to validate the proposed prognostic markers, highlight tumor margins, better map receptor expression profiles of prostate neoplasms, determine the number of 'hot' cells for monitoring the efficacy of anti-cancer therapy which the individual is undergoing and gauge the potential/progression to aggressive PCa; all contributing to improved staging and possibly, survival. Furthermore, quantification of GPR55 expression through confocal analysis of T1117-doped NP-based fluorescence would allow investigation of any association

between GPR55 expression and cancer remission, relapse or resistance following treatment with various chemotherapeutic inhibitory agents.

Although literature states about the emerging research and clinical development trends of BODIPY dye-encapsulated NPs, to our knowledge, the design, synthesis, and characterization of T1117-doped NPs to study receptor expression profile has never been reported. Furthermore, to realize two-color imaging, the dyes can be incorporated simultaneously at a controlled doping ratio into the NPs with switchable emitting wavelengths to achieve minimal background signal by employing an appropriate excitation light source and appropriate excitation/emission filter sets. By using these NPs, one can envision a dynamic, dual-color, co-localization methodology to follow receptors within PCa biopsies. Moreover, the fluorescence-based tissue assay may provide promising results in prognosis due to the technological advantages associated with sensitivity, accuracy, signal amplification, high spatial resolution and improved signal-to-noise ratio. The development of these novel NPs has been focused on its clinical applications as a prognostic tool for accurate prognostic assessment of PCa due to its potential utility in the identification of candidate prognosticators associated with disease status. Noteworthy, this approach can prove useful in predictive oncology in PCa, such as *ex vivo* biomarker profiling, which can provide enormous amounts of prognostic information and help in substantiating appropriate drug, its dosing regimen and outcome for a particular (chemotherapeutic) treatment.

Enhanced understanding of the molecular complexity of PCa and expansion of investigative efforts driven by cleverly designed sophisticated nanotechnology-based image analyses methods in tissue biopsy samples or microarrays from treated and untreated PCa patients, may result in more accurate patient stratification for risk to dictate appropriate therapy and effective management, ultimately contributing to a strong beneficial impact on patient survival.

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