Discovery and Longitudinal Measurement of Candidate Biomarkers of Biochemical Recurrence in Prostate Cancer Patients Treated with CHRT

C. Tonny; D. Doherty; C. O’Shea; B. Morrissey; L. Staunton; B. Flalley; A. Shannon; J. Armstrong and SR. Pennington

1UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin,
2St. Luke’s Hospital, Rathfarnham, Co. Dublin

Summary

In this study, patient samples obtained as part of an on-going clinical trial (ICORG06-15) of Combined Hormone and Radiation Therapy, were used to identify and evaluate a potential serum protein signature of disease recurrence.

A panel of 41 potential candidate biomarker proteins was measured longitudinally in patients with biochemical recurrence and their time matched controls.

1. Introduction

Around one in twelve men in Ireland (www.ncrli.ie) and most of the Western world are diagnosed with prostate cancer (PCa) each year and it is the second leading cause of cancer-related death in men [1]. Generally, prostate cancer is treated effectively with radical prostatectomy, androgen-deprivation therapy, radiotherapy, cryotherapy or a combination of these treatments. Compared with hormonal therapy alone, combined hormone and radiation therapy (CHRT) gives improved disease survival outcome for patients with prostate cancer [2]. However, a significant number of CHRT patients still succumb to recurrent disease. Biochemical recurrence is the earliest indication of treatment failure and is diagnosed as two successive PSA measurements 2ng above a nadir i.e the levels of PSA following radical prostatectomy. However, PSA is considered a poor biomarker as the rise of the PSA level is not always associated with subsequent disease recurrence and treatment failure can therefore occur without a notable increase in the PSA level. Earlier prediction and monitoring of individual patient response to CHRT would allow for more effective clinical decision making and hence personalized treatment [3,4]. Here, serum samples from patients diagnosed with biochemical recurrence (n=3) and time matched controls (n=3) were used for LC-MS/MS-based discovery and longitudinal multiple reaction monitoring (MRM)-based evaluation of potential serum protein biomarkers of treatment failure (Figure 1).

2. Aims and Objectives

The objectives of this research were:

- To apply LC-MS/MS technology for the discovery of novel biomarkers of biochemical recurrence
- To develop MRM assays for a panel of potential candidate biomarkers of biochemical recurrence
- To conduct longitudinal measurements of a candidate protein biomarker panel over the course of patient treatment with CHRT

3. Methods

Patient samples were collected as part of a non-interventional clinical trial in St. Luke’s Hospital, Dublin for PCa patients undergoing treatment with CHRT (ICORG 06-15). Label-free LC-MS/MS based protein discovery was undertaken using MARS14 depleted serum samples as described previously [3]. Samples were analysed on a Thermo Scientific Q-Exactive mass spectrometer with protein identification and quantification undertaken using PEAKS (version no.6) and MaxQuant (version no.1.4.1.2) software. MRM assays were designed using Skyline software (version no.2.5.0.6157). Assays were run with nanoflow reverse phase C18 chromatographic ChipCube-based separation coupled to an Agilent 6460 triple quadrupole mass spectrometer.

A final MRM method consisting of 4 transitions each for 59 peptides correlating to 41 proteins was developed. In total, 85 serum samples taken from 6 patients at multiple time points over the course of treatment with CHRT were used for longitudinal evaluation of the candidate biomarker panel (Figure 3). Patient samples were analysed in 4 batches of 24 samples. Each batch included 5 technical replicates to ensure optimal instrument performance throughout the experiment.

4. Results

Serum samples taken from patients at baseline and time of biochemical recurrence were used for biomarker discovery. 347 proteins were identified following LC-MS/MS analysis of depleted serum on a Q-Exactive mass spectrometer. Overall, 104 proteins showed a significant change between patients with biochemical recurrence and respective controls. 65 of these proteins were identified as potential candidate biomarkers. MRM assays for a panel of 41 proteins were developed and measured in longitudinal patient samples taken from diagnosis until a recent most sample collection (Figure 2).

5. Conclusions

The PCa biomarker candidates discovered as part of this study have been combined with previous candidates for development of a putative serum protein signature of disease recurrence in PCa patients. The data collected from evaluation of longitudinal biomarker expression in patient samples, is currently undergoing extensive analysis. With further validation studies, using samples from additional clinical trials, we anticipate that this study will lead towards the development of a clinically significant biomarker assay to support measurement of patient response to CHRT and prediction of disease recurrence.

References