Cancer Letters 369 (2015) 289–297

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Proteomics discovery of radioresistant cancer biomarkers for radiotherapy

Lei Chang [a,b], Peter Graham [a,b], Jingli Hao [a,b], Joseph Bucci [a,b], David Malouf [c], David Gillatt [c,d], Yong Li [a,b,*]

[a] Cancer Care Centre, St George Hospital, Kogarah, NSW 2217, Australia
[b] St George and Sutherland Clinical School, Faculty of Medicine, UNSW, Kensington, NSW 2052, Australia
[c] Department of Urology, St George Hospital, Kogarah, NSW 2217, Australia
[d] Australian School of Advanced Medicine, Macquarie University, NSW 2109, Australia

ARTICLE INFO

Article history:
Received 9 July 2015
Received in revised form 8 September 2015
Accepted 23 September 2015

Keywords:
Cancer
Proteomics
Mass spectrometry
Biomarker
Radioresistance
Radiotherapy

ABSTRACT

Radiotherapy (RT) is one of the most important strategies in cancer treatment. Radioresistance is a major challenge to RT and results in locoregional recurrence and metastasis. Thus, there is a great interest in investigating biomarkers to distinguish radiosensitive from radioresistant (RR) cancer patients. The development of proteomic techniques has sparked new searches for novel proteins for cancer biomarker discovery. Modern proteomic techniques allow for a high-throughput analysis of samples with the visualization and quantification of thousands of potential protein and peptide markers. The discovery of RR biomarkers can provide a clue for predicting RT response and discover therapeutic targets for developing personalised medicine of individual patients.

In the past decade, emerging advanced proteomic technologies have been performed to identify radiation-related biomarkers in human cancers. This review discusses the mass spectrometry (MS)-based proteomic techniques in RR cancer biomarker discovery, summarises RR biomarkers identified in cancers from proteomics-based findings and explores potential values of RR biomarkers for future clinical trials.

Introduction

Cancer remains one of the leading causes of death which perplexes public health in the world. In 2012, around 14.1 million new cancer cases and 8.2 million cancer deaths occurred in worldwide according to GLOBOCAN estimates [1]. Radiation therapy (RT) is one of the most important strategies to kill cancer cells and shrink tumour. Approximately 50% of all patients with cancer receive RT at some point in their treatment, alone or in combination with surgery and/or chemotherapy [2]. Radioresistance and recurrence are major obstacles for the long-term survival of patients undergoing RT [3,4]. The mechanisms of cancer radioresistance are very complicated and affected by many factors, which severely affect radiation efficacy. One possible reason for RT failure may be the intrinsic radioresistance (primary radioresistance) of a subpopulation of clonogenic cells within the tumour [5] while another reason could be the acquired radioresistance during RT [6]. Understanding the mechanisms of radioresistance and identification of radioresistant (RR) biomarkers are important for the improvement of RT.

A biological marker (biomarker) is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses [7]. The identification of cancer RR biomarkers allows the potential of either selecting alternative treatment modalities or, at least, planning RT in combination with specific radiosensitizer agents to avoid the side-effects. If the biomarkers associated with an individual cancer patient can be identified and potential targets for radiosensitization are found and further validated, it will achieve more favourable therapeutic outcomes in clinics. Therefore, studying RR biomarker is important for predicting the tumour radiosensitivity, planning the best treatment strategy and developing personalised medicine in cancer RT.

Due to the key roles of protein functions, proteomics has become the principal technology for the study of global expression of proteins (biomarkers) in the post-genomic era. It can be applied to cells, tissues or biological fluids, and offer the opportunity to revolutionise biomarker discovery and the development of future medicine. Although genomic and transcriptomic approaches have been applied to study cancer radioresistance, the results do not best reflect the whole protein profiles which are the major functional substance in

* Corresponding author. Tel.: +61 2 91132514; fax: +61 2 91134044.
E-mail address: y.li@unsw.edu.au (Y. Li).

http://dx.doi.org/10.1016/j.canlet.2015.09.013
0304-3835/

cancer cells [8]. Proteomic approaches have not only enabled the identification of thousands of differentially expressed proteins in the complex mixtures of disease and normal samples but also ushered the capability of discriminating disease subtypes/aggressiveness that are not recognised by traditional methods.

Advances in proteomics, especially in mass spectrometry (MS) have rapidly changed our knowledge of biomarker proteins which have simultaneously led to the identification and quantification of thousands of unique proteins and peptides in a complex biological fluid or cell lysate [9]. In association with liquid chromatography or other fractionation techniques, this technique provides molecular information that cannot be gained from gel based techniques alone such as analysing proteins with extreme molecular mass/pI, targeting poorly abundant peptides and proteins, addressing post-translational modifications (PTMs) [10]. MS, coupled with technologies for sample fractionation and automated data analysis, provides a platform to identify protein expression differences associated with cancer radioresistance in complex biological samples [11]. Proteomic technology presents the benefit that it can develop the whole proteome of RR cancer cells, reflect the functions of proteins, establish biomarker interconnection, and discover predictive therapeutic proteins [12]. Therefore, proteomic techniques offer an ideal platform for identification and quantification of novel RR proteins in predicting therapeutic outcome, identifying potential therapeutic targets and developing individualised treatment regimen to overcome radioresistance.

Lacombe et al. recently reviewed the main characteristics of tumour radioresistance and normal tissue radiosensitivity and described the existing predictive assays to predict RT outcomes with the proteomics studies [8]. Skvortsov et al. also reviewed proteome-based identification of novel biomarkers to predict tumour radiation response protein in profiling of cancer stem cells (CSCs) and radiation resistance [13]. The MS-based proteomic techniques in cancer RR studies have not been reported before. This review focuses on MS-based proteomic techniques in RR cancer biomarker discovery, summarises RR cancer biomarkers identified by proteomic techniques and explores their potential values for future clinical trials.

MS-based proteomics techniques in cancer RR biomarker discovery and validation

During the past few years, accumulating number of MS proteomics studies have been applied to identify potential biomarkers associated with cancer radioresistance. These proteomics techniques consist of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), isobaric tags for relative and absolute quantitation (iTRAQ), liquid chromatography-tandem mass spectrometry (LC-MS/MS), multiple reaction monitoring (MRM) as well as Sequential Window Acquisition of all THeoretical Mass Spectra (SWATH-MS). Here we discuss the 5 selected MS proteomics techniques in discovery and validation of cancer RR biomarkers.

Gel-based proteomics

MALDI-TOF-MS is a single proteomic approach, normally applied to analyze relatively simple protein mixtures. The sample is mixed with a solution of an appropriate matrix and allowed to co-crystallise directly on special sample plates [14]. This technology is a useful and effective approach for screening peptide masses of tryptic digests because of requiring relatively less intense sample preparation and facilitating data interpretation with showing on peak in spectrum. It is a high throughput technology and can be used to identify protein or peptide profiles. It is very suitable for screening studies. There are several kinds of the MALDI-TOF-MS instruments available such as 5800 MALDI-TOF-MS Analyzer from AB Sciex and ultrafleXtreme from Bruker (USA).

MALDI-TOF-MS has been used in a number of studies for the diagnosis and treatment of human cancers including prostate [15,16], breast [17,18], lung [19] and other cancers [20,21]. Two-dimensional gel electrophoresis (2DE) coupled to MALDI-TOF-MS is frequently applied for comparative proteome analysis in the discovery of biomarkers [22]. Zhong et al. used 2DE combined with MALDI-TOF-MS to isolate and identify membrane proteins in PANC-1 pancreatic cancer cells [23]. However, only a few studies were reported to utilise the MALDI-TOF-MS approach to identify and verify RR biomarkers in cancers. Liang et al. found the interaction sites between 1,2,5-selenadiazole and the model peptide of redox enzyme thioredoxin reductase (TrxR) by MALDI-TOF-MS, clearly demonstrating TrxR as a potential target for therapy of human RR melanoma cancer [24]. Wei et al. used 2DE combined with MALDI-TOF-MS to identify differentially expression proteins (DEPs) related to radioresistance or multiple drug resistance (MDR) using human lung adenocarcinoma (HLA) A549 cells and cisplatin-resistant A549/DDP cells after irradiation, in order to evaluate whether the MDR can elevate radioresistance [25]. In this study, 27 DEPs were identified between A549 and A549/DDP cells and functionally divided into 6 categories composed of metabolic enzymes, signal transduction, detoxification or translation, chaperones, cellular structure proteins, calcium-binding proteins [25]. Among these identified proteins, 4 DEPs including HSPB1, Vimentin, Cofilin and annexin A4 were further validated in A549 and A549/DDP cells and lung adenocarcinoma tissue by western blotting (WB) and immunohistochemistry (IHC), respectively. The results further confirmed that these 4 DEPs were associated with MDR as well as radioresistance and were potential biomarkers for predicting HLA response to MDR and radioresistance. In another study, using 2DE combined with MALDI-TOF-MS approach, Li et al. demonstrated that Prx-1 could be used as a potential therapeutic target for enhancing the tumour response to radiation [26].

To identify RR proteins, 17 patients who suffered from a locally advanced adenocarcinoma of the rectum were recruited and tumour tissues were obtained before or after radiation. It was found that 10 patients demonstrated an effective response to RT, whereas 7 showed radioresistance. Using 2DE/MALDI-TOF-MS analysis, 5 proteins including Tropomodulin, HSP 42, β-tubulin, annexin V and calsenilin were found to be up-regulated while 3 proteins including keratin type I, a notch 2 protein homologue and the DNA repair protein RAD51L3 were down-regulated in radiosensitive tumours, indicating that the mechanisms of radioresistance are strongly associated with the activation of DNA damage and repair, cell cycle as well as apoptosis in rectal cancer and the identified markers could be used as a basis for developing an assay for testing rectal cancers for radioresistance [27].

The advantages of MALDI-TOF-MS are that it is automated, with high sensitivity and low cost. This technique is multidimensional and gives absolute mass measurements and works well with large polypeptides (>30 kDa). The main disadvantage of MALDI-TOF-MS is its sensitivity to contaminants such as salts, therefore, reproducibility of results may be a problem. In addition, the real protein markers cannot be identified using this technique. As modern MS techniques are emerging, this technique is much less used for biomarker discovery in cancers including RR biomarkers.

iTRAQ

iTRAQ technology is a shotgun based quantization technique using the instruments such as Q-Trap instrument from SCIEX (USA) and also referred to as a bottom up approach which allows the concurrent identification and relative quantification of hundreds of proteins in up to 8 different biological samples in a single experiment [28].

iTRAQ based quantitative proteomics is a promising approach for global comparison of protein expression in relatively small amounts of samples. This labelling strategy ensures no loss of information from samples involving PTM such as the scrutiny of signal transduction pathways that often involve phosphorylation phenomena [29]. In addition, the multiplexing capacity of these reagents allows for information replication within certain LC-MS/MS experimental regimes, providing additional statistical validation within any given experiment. Cai et al. identified 54 proteins with differential expression in nasopharyngeal carcinoma (NPC) and the adjacent non-tumour tissue by iTRAQ coupled with two-dimensional LC-MS/MS, and these identified proteins were further validated by qRT-PCR and WB in NPC tissues compared to normal nasopharyngeal tissues [30].

iTRAQ is ideally suited for biomarker discovery as it provides both relative quantification and multiplexing in a single experiment and has been applied to the analysis of clinical samples [31,32] and *in vitro* cell study [33]. In one study, it was used to investigate the RR biomarkers in breast cancer (BC) cell lines (MCF7/MCF7RR, MDA-MB-231/MDA-MB-231RR, T47D/T47DRR) where 40 potential biomarkers were identified [34]. Using iTRAQ, another study reported that up-regulation of the nonhomologous end-joining (NHEJ) pathway which is critical for DNA repair of irradiated cells is involved in radioresistance of hypoxic epithelial carcinoma cells A431 cells [35]. This technique is useful for identifying and quantifying proteins across diverse molecular weight (MW) and isoelectric point (pI) ranges, functional categories, cellular locations and abundances. However, the disadvantages of this technique are that it is very time consuming, extremely laborious and very expensive.

Label-free LC-MS/MS

LC-MS/MS (LC-based separation techniques directly coupled to automated MS/MS) strategies offer high-throughput analyses resulting in the acquisition of hundreds of thousands of MS/MS fragmentation spectra in a single experiment [36]. The label-free LC-MS/MS method using the instruments such as Orbitrap Velos from Thermo Electron, (USA) provides protein quantification by comparing MS measurements of different samples. Label-free quantification through spectral counting is based on the principle that highly abundant peptides will generate a higher number of MS/MS spectra [37]. It is a powerful technique that can be sensitively and selectively performed in many applications such as protein profilling in human cancers [38,39]. The LC-MS/MS approach was applied to analyze global proteins present in BC cell lines T47D and T47DRR, and a total of 586 and 652 proteins were identified in T47D and T47DRR cells, respectively [34]. This approach was also performed to investigate the underlying molecular mechanisms for gemcitabine resistance in pancreatic cancer [40]. In this study, a total of 1931 proteins were identified and 787 DEPs were quantified in the pancreatic cancer cell lines BxPC3, PANC-1, and HPDE [40]. Zeng et al. performed a global lung cancer serum biomarker discovery study using LC-MS/MS in a set of pooled non-small cell lung carcinoma (NSCLC) sera and matched controls, and identified 49 differentially abundant candidate proteins [41]. Yang et al. showed that using LC-MS/MS method, 265 distinct glycoproteins were confidently identified in urinary samples obtained from bladder cancer patients, providing novel biomarkers for the early detection [42].

The LC-MS/MS approach has been recently applied to investigate human cancer radioresistance [43]. In one study, a total of 36 DEPs were identified from RR and radiosensitive (control) astrocytoma patients using 2D-LC-MS/MS approach and two markers-cofilin-1 and phosphoglycerate kinase 1 (PGK1) were found to be significantly up-regulated in RR astrocytomas [44], indicating these markers are associated with astrocytoma radioresistance and have potential to be used as therapeutic targets. Our recent results showed that totally 309 signalling pathway proteins were identified to be significantly different between prostate cancer (CaP) RR (PC-3RR, DU145RR and LNCaPRR) and parental CaP (PC-3, DU145 and LNCaP) cells using the label-free LC-MS/MS method. Nineteen of them are overlapped among three paired CaP cell lines and associated with CaP metastasis, progression, and radioresistance (unpublished data). The work flow of LC-MS/MS proteomics technique for CaP-RR biomaker discovery and validation of identified potential biomarkers is shown in Fig. 1.

LC-MS/MS has seen enormous growth in clinical laboratories in the last 10–15 years because it offers analytical specificity superior to that of immunoassays or high performance liquid chromatography (HPLC) for low MW analytes. However, as large amounts of information are obtained, it is time consuming to analyze. LC-MS/MS is not suited for routine clinical analysis and the separation of larger molecules and analytes covering a broad range of size and hydrophobicity.

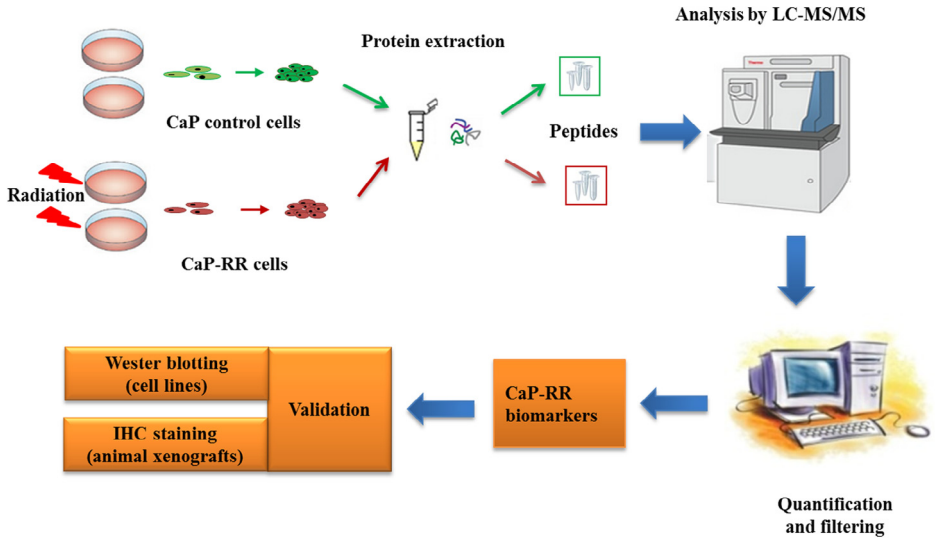

Fig. 1. The work flow of LC-MS/MS proteomics technique for CaP-RR biomarker discovery and validation. CaP-RR and CaP parental control cells were prepared for protein extraction and analysed by LC-MS/MS. After quantification and filtering, the potential CaP-RR biomarker candidates identified were validated on CaP-RR cell lines and CaP-RR animal xenograft tissues using WB and IHC staining, respectively.

MRM

MRM (also called selected reaction monitoring (SRM)) is a highly specific and sensitive label-free technique for quantifying targeted protein/peptides abundances in complex biological samples. It refers to a tandem MS (MS/MS) scan mode that is coupled with triple quadrupole or hybrid quad/trap MS instrumentation. It is commonly used for the analysis of small molecules. The MRM proteomics technology allows for targeted analysis of proteins of interest while all other proteins are filtered out. It is a promising method used in tandem MS for protein quantitation and validation in a wide variety of clinical samples [45,46].

MRM can test a large number of potential biomarkers and is a suitable method instead of other validation approaches such as WB, IHC [47]. MRM can test a large number of potential biomarkers and is a suitable method instead of other validation approaches such as WB, IHC etc [47]. With MRM-based approach, together with the use of isotope-coded ATP-affinity probes, 24 and 13 of the quantified kinases were significantly up- and down-regulated in MCF-7/C6 compared to MCF-7/WT cells, respectively. In addition, key kinase modulators involved in ERK (25), Toll-like receptor (TLR) (10), and ErbB (8) pathways were successfully quantified [48]. Ren et al. used LC-MS/MS-MRM as well as WB to confirm the up-regulation of Ku70/Ku80 dimer DNA repair, glycolysis, integrin, glycoprotein turnover and STAT1 pathways perturbed by hypoxia in A431 epithelial carcinoma cells [35], demonstrating that the MRM results were consistent with their previous iTRAQ results and that hypoxia induced several biological processes involved in tumour migration and radioresistance.

MRM technology has the potential to supplant ELISAs and other immunoassays for biomarker verification as the time and cost of designing MRM assays is far less than that for the traditional methods that employ antibodies. MRM also becomes possible to probe for several predicted phosphopeptides from a known protein sequence. This differs significantly from other MS techniques for identifying phosphopeptides [49]. However, MRM also has some limitations. Like many immunoglobulin sequences, the proteins may be too short and variable to produce candidates [50]. Another disadvantage is that genetic variants in the selected peptide may prevent the determination by MRM [50].

SWATH-MS

SWATH-MS is an emerging proteomic approach in which data are acquired on a fast, high resolution Q-orbitrap or tripleTOF mass spectrometer by repeatedly cycling through sequential isolation windows over the whole chromatographic elution range [51]. It provides multiplexed quantitative MS/MS information for all peptides ionizing in a sample in an unbiased manner and then uses spectral libraries to interrogate these spectra for identification and quantification of a priori peptides. This approach allows rapid and higher throughput verification and validation of marker candidates from samples, and provides complementary evaluation of the protein profile. In comparison to the data-dependent acquisition (DDA) method, SWATH-MS is based on a DIA (data independent acquisition) mode and it outperformed the DDA method in its quantification ability and less signal variation; additionally the number of quantified peptide is markedly increased [52]. SWATH-MS method features both the global screening capabilities of discovery based proteomics, and the sensitivity of SRM by activating all peptides eluting in real time within the predefined windows and multiplexed recording of all fragment ions. This new technique has been successfully applied for the identification of biomarkers for aggressive CaPs using clinical tumour tissues, by searching against established glycoprotein maps [53]. In this study, 2 biomarkers out of 220 differentially expressed glycoproteins were discovered and further validated to be associated with aggressive CaP [53]. This finding may assist in stratifying CaP and avoiding overtreatment of non-aggressive CaP. In another study, by combining shotgun discovery proteomics-iTRAQ with SWATH-MS, Zhang et al. found that over-expression of CD109 is significantly associated with NSCLC [54]. All the findings support this new technique is promising for identification and validation of RR cancer biomarkers in the future study.

RR biomarkers in cancers

Many potential RR cancer biomarkers have been identified by different proteomics approaches. These identified markers encompass a variety of roles including cell cycle, DNA repair, metabolism, signal transduction. In this section, we focus on discussing discovery of RR biomarkers in cancer cell lines by different proteomic approaches and validation of identified potential biomarkers by WB or IHC. The RR biomarkers identified by proteomics in different cancers are summarised in Table 1. Among these identified putative RR biomarkers, 18 of them were previously reviewed [8] and the new markers which were not discussed are specifically highlighted with the bold font in Table 1.

Head and neck cancer

Head and neck cancer (HNC) is a cancer that starts in the lip, oral cavity (mouth), nasal cavity (inside the nose), paranasal sinuses, pharynx, and larynx, 90% of which is squamous cell head and neck cancers (SCHNC) [66]. SCHNC is the sixth leading cancer by incidence worldwide and eighth by death [67]. The five-year survival rate of patients with SCHNC is around 40–50%. Despite RT is the effectively main treatment for HNC, radioresistance causes tumour recurrence and remains an unsolved problem [68]. Thus, identifying specific biomarkers associated with radioresistance will help improve treatment outcome in HNC patients.

Currently, most of reported proteomics-based RR biomarker studies are in HNC. Several proteomics studies related to NPC radioresistance have recently been reported [55,57]. Feng et al. first established a RR subclone cell line (CNE2-RR) derived from NPC cell line CNE2 by fractioned radiation treatment and compared the protein expression profiles of CNE2-RR and CNE2 cell lines by 2DE method [55]. They found that total 34 differential proteins were identified to have significant differences. Among them, 14-3-3σ and Maspin were down-regulated while GRP78 and Mn-SOD were up-regulated in the CNE2-RR cells compared with CNE2 cells, which was confirmed by WB and IHC performed on 39 RR and 51 radiosensitive human NPC tumour biopsies. In the following functional study, up-regulation of 14-3-3σ restored the radiosensitivity of the CNE2-RR cell line, indicating that down-regulation of 14-3-3σ could play an important role in the development of NPC radioresistance [55]. Zhang et al. reported the differential proteins in NPC using CNE1-RR and CNE1 cell lines by 2DE/MALDI-TOF-MS approach [56]. They found that 13 differential proteins were detected and HSP27, as one of up-regulated proteins in CNE1-RR cells, was further investigated by several experiments. It was reported that 88 NPC patients including 42 RR and 46 radiosensitive patients who were treated by curative-intent RT (a total dose of 70 Gy) using a modified linear accelerator were recruited [57]. NPC RR tissues were compared with radiosensitive tissues using 2DE/MALDI-TOF method and 12 differential proteins were identified to be involved in radioresistance. ERp29 was found to be significantly up-regulated in NPC RR tissues and further investigated by IHC, shRNA assay, flow cytometry. Li et al. also reported that 16 DEPs were identified in NPC CNE2-RR cell line compared to CNE2 cell line by 2DE/MALDI-TOF analysis, demonstrating among the identified proteins, Nm23 H1 was significantly increased while annexin A3 was significantly down-regulated in CNE2-RR cells [58].

Table 1
Putative RR biomarkers identified in 4 cancers by proteomics approaches.

Putative biomarker	Source	Radiation dose	Proteomics method	Validation method	Reference
14-3-3σ(↓), Maspin (↓), GRP78 (↑), Mn-SOD (↑)	Nasopharyngeal cancer cell line CNE2	Total 11 Gy (single dose)	2DE	WB and IHC	[55]
HSP27(↑)	Nasopharyngeal cancer cell line CNE1	Total 13 Gy (single dose)	2DE/MALDI-TOF	WB	[56]
ERp29(↑)	Nasopharyngeal cancer tissues from patients*	Total 70 Gy	2DE/MALDI-TOF	IHC, shRNA assay and FACS	[57]
Nm23 H1(↑), annexin A3(↓)	Nasopharyngeal cancer cell line CNE2	Total 64 Gy (4 Gy/16 times for 1 year)	2DE/MALDI-TOF	WB	[58]
Gp96(↑), Grp78(↑), HSP60(↑), Rab40B(↑), GDF-15 (↑), annexin V(↓)	Oral epidermoid carcinoma cell line KB, tongue squamous cell carcinoma cell line SAS, gingival epidermoid carcinoma cell line OECM1	Total 60 Gy (2 Gy/ per time)	2DE/MALDI-TOF	RT-PCR and xenografted mouse tumour study	[59]
CLIC1(↓)	Laryngeal cancer cell line Hep-2	Total 60 Gy (2 Gy per fraction, two times per week for 15 weeks).	2DE/MALDI-TOF	WB and IHC	[60]
Rac1(↑)	Head and neck squamous cell carcinoma cell lines FaDu and SCC25	Total 100 Gy (10 Gy ten times every two weeks)	2DDIGE/MALDI-TOF/TOF	WB	
NM23-H1(↑), PA2G4(↑)	Head and neck squamous cell carcinoma cell lines QLL1, SCC15 and SCC25	Total 60 Gy (2 Gy/per time)	MALDI-TOF	WB	[61]
Peroxiredoxin II (↑)	BC cell line MCF-7	Total 60 Gy (2 Gy/five times per week for 6 weeks)	2DE/MS	WB	[62]
The S26S proteasome(↓), GRP78 (↓)	BC cell lines MCF-7, MDA-MB-231and T47D	Total 40 Gy (2 Gy per week for 20 weeks)	2DE/MS, LC-MS/MS and quantitative iTRAQ	WB and IHC	[34]
CTSD (↑), GSN (↑), MRC2 (↑)	BC cell line MDA-MB-231	Total 10 Gy	SILAC-based quantitative proteomics	WB	[63]
CHK1(↑), CDK1(↑), CDK2(↑)	**BC** cell lines MCF-7	Total 30 Gy (2 Gy/five times per week for 3 weeks)	LC-MRM	WB	[48]
NME1(↑), HSPA8(↑), APEX1(↑), PAI-RBP1 (↑)	CaP cell lines PC-3, DU145 and LNCaP	Total 10 Gy (2 Gy for 5 days)	2DIGE/MALDI-TOF	WB	[64]
ALDOA(↑)	CaP cell lines PC-3, DU145 and LNCaP	Total 10 Gy (2 Gy for 5 days)	LC-MS/MS	WB and IHC	Unpublished results
HSPB1 (↑), annexin A4 (↑), Cofilin l (↑), Vimentin (↑)	Lung cancer cell line A549	Total 6 Gy	2DE/MALDI-TOF-MS	WB and IHC	[25]
ATP (↑), HMGB-1(↑)	Lung cancer cells derived from patients*	Single 18 Gy or 2 Gy for 4 times	LC-MS/MS	WB and ELISA	[43]
α1-AT (↑)	Lung cancer specimen*	A total dose of 62.4–68.0 Gy	2DE and LC-MS/MS	ELISA	[65]

Notes: ↑ indicates increased expression. ↓ indicates decreased expression. * indicates the samples from human tissues. Bold font indicates that the biomarkers were not discussed in previous published reviews. FACS: flow cytometry; IHC: immunohistochemistry; ShRNA: short hairpin RNA; WB: western blotting.

Proteomic studies on radioresistance were also reported in other HNCs. Lin et al. studied three RR HNC cell lines including KB cell line (an oral epidermoid carcinoma), SAS cell line (tongue squamous cell carcinoma) and OECM1 cell line (gingival epidermoid carcinoma) compared to their parental cell lines by 2DE/MALDI-TOF [59], and found that 64 proteins were identified to be potentially associated with radioresistance which were involved in several cellular pathways including regulation of stimulus response, cell apoptosis, and glycolysis [59]. Among the identified proteins, Gp96, Grp78, HSP60, Rab40B, and GDF-15 were up-regulated while annexin V was down-regulated in RR HNC cell lines. Further investigation showed that Gp96-siRNA (small interfering RNA) transfectants displayed a radiation-induced growth delay, reduction in colonogenic survival, increased cellular reactive oxygen species (ROS) levels and proportion of the cells in the G2/M phase [59]. Xenograft mice administered with combination of Gp96-siRNA and RT showed significantly enhanced tumour growth suppression in comparison with RT alone [59].

To identify the DEPs in RR laryngeal cancer, HEp-2-RR and HEp-2 cell lines were compared by 2DE/MALDI-TOF and 16 proteins showed significantly altered expression levels [60]. Among the identified markers, the potential marker-chloride intracellular channel 1 (CLIC1) was found to be associated with laryngeal cancer radioresistance via inhibition of ROS production in the functional study [60]. Skvortsov et al. used 2DDIGE followed by MALDI-TOF-MS to investigate differential proteins between SCHNC RR cell lines (FaDuRR and SCC25RR) and its parental cell lines (FaDu and SCC25) and found 45 proteins were modulated in FaDuRR and SCC25RR cells compared to parental cells, which were closely related to cell migration regulated by Rac1 protein, indicating that Rac1 protein could be considered as a new therapeutic target RR SCHNC [69]. Lee et al. identified 51 proteins with commonly altered expression in SCHNC RR cell lines (QLL1, SCC15 and SCC25) using the 2D SDS-PAGE proteomics approach, 18 of which were cancer-related proteins [61]. Among these identified cancer markers, the NM23-H1 protein was further validated in SCHNC RR cell lines by WB with increased expression, suggesting that this marker is a reliable predictor for RR oral cancer [61].

All these studies support that many proteins are associated with HNC radioresistance and these proteins have potential for predicting RT response and improving HNC response to RT in clinics.

Breast cancer

BC is the most prevalent malignancy in women and the second leading cause of cancer-related deaths in developed countries. RT is widely used as a part of a tri-modal treatment with chemotherapy and surgery; however, approximately 50% of BC patients have

experienced malignant microfoci scattered throughout the breast tissue that can easily progress to metastatic BC [70]. Radioresistance has been identified as a factor that limits the effectiveness of RT in the treatment of BC. Therefore, discovery of RR biomarkers is important for BC RT.

Wang et al. first identified 100 DEPs involved in BC radioresistance by comparing RR MCF+FIR3 and radiosensitive MCF+FIS4 BC cell lines using 2DE/MS method [62]. Among the identified potential proteins, peroxiredoxin II (PrxII) which plays an important role in the redox process was found to have 4-fold increase in MCF+FIR3 RR cells compared with MCF+FIS4 radiosensitive cells. Knock down of PrxII using siRNA could improve radiosensitivity while overexpression of PrxII resulted in BC radioresistance, indicating that ROS is critical for BC radioresistance and that stress-induced overexpression of PrxII increased radioresistance via protection of cancer cells from radiation-induced oxidative damage [62]. Another study also compared three RR BC cell lines (MCF7RR, MDA-MB-231RR and T47DRR) with their parental cell lines using three proteomics methods including 2DE/MS, LC-MS/MS and iTRAQ to identify predictive biomarkers of radioresistance [71]. In 2DE/MS analysis, 50 proteins were identified with significant differences in one or more BC cell lines. LC-MS/MS approach was used as a complementary approach to 2DE-MS for the analysis of all proteins present in T47D and T47DRR cells, showing overall, 242 unique proteins were identified in T47D cells and 310 unique proteins were identified in T47DRR cells. In quantitative iTRAQ, 40 proteins were detected and showed quantitatively different expression levels between these three RR cell lines and parental cell lines. However, there were very few overlapping identified proteins from the data produced through the 2DE/MS, LC–MS/MS and iTRAQ approaches, suggesting different proteomic techniques have different advantages. Among the identified proteins, both 26S and GRP78 markers were found to be down-regulated in all RR cell lines compared with their parental cell lines by WB [71]. Using the stable isotope labelling by amino acids in cell culture (SILAC)-based proteomic analysis, Kim et al. investigated the cytosolic proteins produced by irradiated MDA-MB-231 BC cells treated with a single or fractionated 10 Gy dose of 137Cs γ-radiation and found a number of tumour-derived factors (CTSD, GSN, and MRC2) were upregulated, indicating that these enhanced factors are promising targets for modulation of the immune response during radiation treatment [63]. In a recent study, Guo et al. assessed the global kinome of RR MCF-7/C6 and their parental MCF-7 BC cell lines by LC-MRM method, and found that 24 and 13 of the quantified kinases were significantly up- and down-regulated in MCF-7/C6 relative to the parental MCF-7 cells, respectively. In addition, the checkpoint kinase 1 (CHK1), cyclin-dependent kinases 1 and 2 (CDK1 and CDK2) were found to be overexpressed in RR MCF-7/C6 cells, which were further validated by WB [48], suggesting that DNA repair and cell cycle mechanisms are involved in BC radioresistance.

All findings from BC radioresistance studies may provide new potential targets to sensitise radiation as well as biomarkers to predict radiation sensitivity in human BCs. However, the shortcoming for all three studies is that no identifed markers have been validated in human RR BC tissue samples to evaluate their clinical values.

Prostate cancer

CaP is the most common cancer in men in Western countries. RT is a standard treatment option for both organ-confined and regionally advanced CaP. Despite more and more effective advances in radiation delivery procedures, about 50% CaP patients undergoing RT suffer from relapse (recurrence) within 5 years of treatment [72]. Radioresistance is a major challenge for the current CaP RT. A personalised approach to treatment is urgently needed allowing patients unlikely to benefit from conventional RT to be directed towards hypofractionated RT [73] or other therapeutic options. The identification of CaP-RR biomarkers allows the potential of either selecting alternative treatment modalities or, at least, planning RT in combination with specific radiosensitising agents, avoiding the side-effects.

Using 2DDIGE/MALDI-TOF approaches, Skvortsova et al. compared the protein differences with three CaP-RR cell lines (PC3-RR, DU145-RR and LNCaP-RR) and their parental cells to examine the mechanisms involved in CaP radioresistance [64]. In this study, 27 proteins, which were associated with the regulation of intracellular pathways for cell survival, motility, mutagenesis and DNA repair, were found to express differently between three RR and their paretal cell lines. Five proteins including NME1, HSPA8, APEX1, PAI-RBP1 and RAB11A showed the most significant different overexpression and were further confirmed in CaP-RR cell lines by WB. Furthermore, as an DNA repair associated enzyme, knock down of APEX1 could significantly increase radiosensitivity in CaP. In our recent study, using three established CaP-RR cell lines (PC-3RR, DU145RR and LNCaPRR), we successfully identified 19 protein differences involved in CaP radioresistance using a label-free LC-MS/MS proteomic technique (unpublished data). In addition, one selected important protein ALDOA (Aldolase A, Fructose-Bisphosphate) were further validated in CaP-RR cell lines and PC-3RR s.c. xenografts by WB and IHC (Fig. 2), respectively. Furthermore, the ALDOA was functionally verified in CaP-RR cells using siRNA knock down for increasing radiosensitivity (unpublished data). These findings indicate that mutiple mechanisms regulate radioresistance and targeting identified potential biomarkers may serve as a tool to overcome CaP radioresistance and improve the prognosis of CaP patients with RT.

Lung cancer

Lung cancer is a major globe health problem for men and women. The main primary types of lung cancer are small-cell lung carcinoma (SCLC) and NSCLC. RT is an important adjuvant therapy for curative intent in NSCLC patients who are not eligible for surgery. However, NSCLC commonly develops resistance to radiation. Discovery of potential RR biomarkers for prediction and therapeutic purpose is growing in importance for NSCLC.

It was reported that 2DE/MALDI-TOF-MS was used to identify DEPs related to radioresistance or MDR using HLA A549 cells and cisplatin-resistant A549/DDP cells after irradiation, in order to evaluate whether the MDR can elevate the radioresistance [25]. Additionally, proteomic analyses of the secretome by LC-MS/MS identified a total yield of 978 proteins, comparison of irradiated and non-irradiated cancer-associated fibroblasts (CAFs) derived from NSCLC patients, of which 261 had relevant inflammatory or immunomodulatory functions in irradiated CAFs [43]. To find whether potential serum biomarkers with chemoradiotherapy (CRT) sensitivity can predict clinical outcome upon treatment in NSCLC, Huang et al. analysed the proteins in sera (sensitive group vs CRT resistant group) by 2DE and LC-MS/MS, respectively [65] and demonstrated that 6 proteins were identified in CRT resistant group and Alpha-1-antitrypsin (α1-AT), as one of them, was further validated by ELISA, indicating that the potential biomarkers detected by the proteomic approaches can predict the outcome of treatment in NSCLC patients.

Conclusions and future perspectives

Biomarker research continues to be a developing field. Identification of RR potential biomarkers is very imperative for predicting cancer radioresistance and developing biomarker-guided targeted therapy or combination therapy with the aim of sensitising RT on which new treatments and prevention methods can be developed.

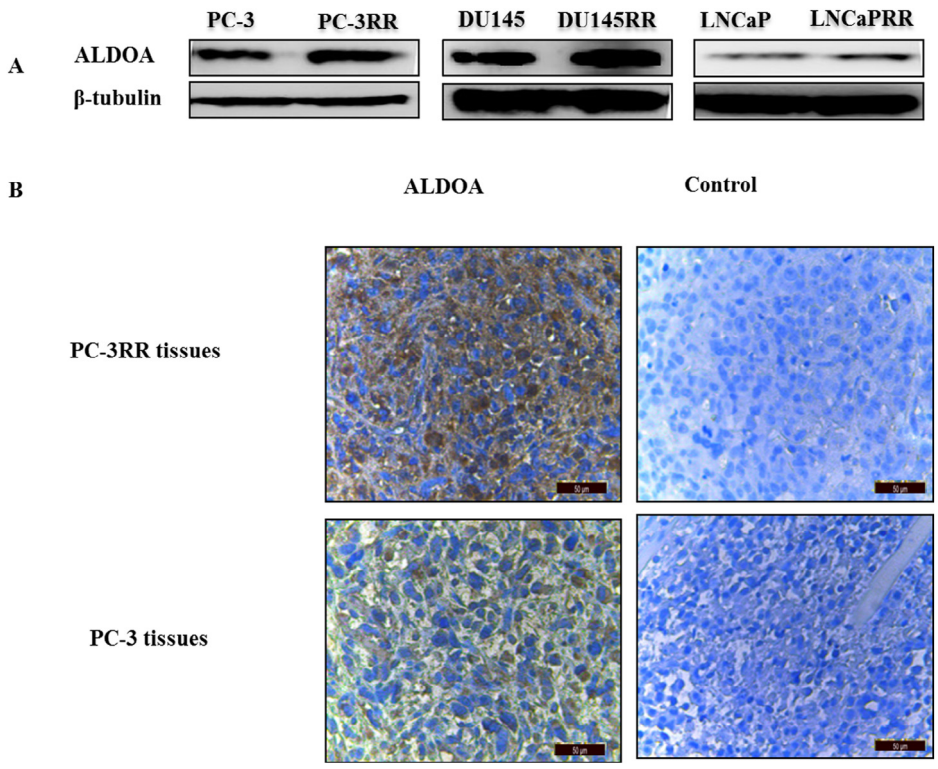

Fig. 2. Validation of ALDOA in CaP-RR cell lines and PC-3RR s.c. xenograft tumours. (A) The expression of ALDOA was increased in CaP-RR (PC-3RR, DU145RR and LNCaPRR) cells compared with CaP (PC-3, DU145 and LNCaP) cells. β-tubulin was used as a loading control. (B) Representative images for the expression of ALDOA in PC-3 and PC-3RR s.c. xenografts using IHC. Strong positive staining for ALDOA was seen in PC-3RR tumour tissues; weak staining was seen in PC-3 tumour tissues; no positive staining was found in control slides. Brown indicates positive staining while blue indicates nuclear staining. Magnification ×40 in all images. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

MS technologies, which can detect thousands of proteins at nanomolar concentrations, have led to the expansion of work in the field of finding markers to diagnose diseases, disease progression during treatment or responsiveness to RT, holding great promise for the detection of RR candidate protein biomarkers for clinical application to improve cancer patients' outcome. The MS-based proteomic approaches are very promising in identifing predictable biomarkers in cancer radioresistance. Several interesting protein biomarkers have been identified to be involved in radiation response.

The studies of RR biomarkers in human cancers using proteomic techniques are emerging research area. While multiple proteomic techniques are well established and used for cancer RR biomarker discovery, there are limitations to each technique. In the future, complementary proteomic techniques should be used to cover low and high MW proteins, over a wide dynamic range to achieve the maximum chance for differential proteins predicting for radioresistance and identifying potential therapeutic targets for innovative therapies. In addition, discovering and verifying cancer biomarkers directly in human samples is tremendously difficult due to considerable genetic, behavioural, and environmental heterogeneity. RR mouse models or other contemporary models such as patients-derived xenografts (PDX) or explanted human tissues from RR cancer patients should be considered for radioresistance biomarker study in the future. Furthermore, standardised protocols for sample processing, data normalisation and clinical result interpretation require further investigation.

Studying RR biomarkers using proteomic approches have been performed in several cancers and the preliminary results are very encouraging. The identified potential protein markers were further validated in human cancer cell lines in most of studies by WB or IHC. However, a large size of human RR tumour tissue samples from cancer patients are required to translate these finding from bench to clinic practice. We also envision that targeted MS experiments that allow for high-throughput multiplexed quantitative analysis will be widely used. Future clinical research designs should consider prospectively incorporating pre-treatment tumour biopsies and biofluid collection during and after the RT course to track RR biomarkers in a temporal manner.

The success in identifying cancer RR biomarkers can guide clinicians in predicting treatment outcome of RT and develop a tailored individual therapeutic regimen to magnify the benefit of RT to cancer patients. Strategies directed to early prediction of RR cancer may be more effective to extend survival of cancer patients rather than attempt to improve the outcome of patients with clinically proven RT failure. Studying the biological functions of these cancer RR biomarkers may reveal the mechanisms of radioresistance and develop biomarker-guided targeted therapy or combination therapy with the aim of sensitising RT on which new treatments and prevention methods can be developed.

Acknowledgements

Our study is supported in part by NHMRC Career Development Fellowship (630664); Cancer Research Trust Fund (Cancer Care Centre, St George Hospital); and Prostate and Breast Cancer Foundation (PBCF) and China Scholarship Council. The authors thank Mr Ken Hopper, Mr Ese Enari, Mr Alex Wallace and Mr Peter Treacy from Cancer Care Centre, Sydney, for their technical support. We also thank Dr Valerie Wasinger from the Bioanalytical Mass Spectrometry Facility (BMSF), Mark Wainwright Analytical centre, UNSW for her excellent assistance in proteomic analysis.

Conflict of interest

No declared conflicts of interest.

References

[1] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global cancer statistics, CA Cancer J. Clin. 65 (2015) (2012) 87–108.
[2] G. Delaney, S. Jacob, C. Featherstone, M. Barton, The role of radiotherapy in cancer treatment: estimating optimal utilization from a review of evidence-based clinical guidelines, Cancer 104 (2005) 1129–1137.
[3] A.C. Begg, F.A. Stewart, C. Vens, Strategies to improve radiotherapy with targeted drugs, Nat. Rev. Cancer 11 (2011) 239–253.
[4] K. Ogawa, Y. Yoshioka, F. Isohashi, Y. Seo, K. Yoshida, H. Yamazaki, Radiotherapy targeting cancer stem cells: current views and future perspectives, Anticancer Res. 33 (2013) 747–754.
[5] T. Wang, L.R. Languino, J. Lian, G. Stein, M. Blute, T.J. Fitzgerald, Molecular targets for radiation oncology in prostate cancer, Front. Oncol. 1 (2011) 17.
[6] L. Chang, P.H. Graham, J. Hao, J. Ni, J. Bucci, P.J. Cozzi, et al., PI3K/Akt/mTOR pathway inhibitors enhance radiosensitivity in radioresistant prostate cancer cells through inducing apoptosis, reducing autophagy, suppressing NHEJ and HR repair pathways, Cell Death Dis. 5 (2014) e1437.
[7] S. Naylor, Biomarkers: current perspectives and future prospects, Expert Rev. Mol. Diagn. 3 (2003) 525–529.
[8] J. Lacombe, D. Azria, A. Mange, J. Solassol, Proteomic approaches to identify biomarkers predictive of radiotherapy outcomes, Expert Rev. Proteomics 10 (2013) 33–42.
[9] J.R. Yates, C.I. Ruse, A. Nakorchevsky, Proteomics by mass spectrometry: approaches, advances, and applications, Annu. Rev. Biomed. Eng. 11 (2009) 49–79.
[10] S. Wolff, A. Otto, D. Albrecht, J.S. Zeng, K. Buttner, M. Gluckmann, et al., Gel-free and gel-based proteomics in Bacillus subtilis: a comparative study, Mol. Cell. Proteomics 5 (2006) 1183–1192.
[11] S.K. Van Riper, E.P. de Jong, J.V. Carlis, T.J. Griffin, Mass spectrometry-based proteomics: basic principles and emerging technologies and directions, Adv. Exp. Med. Biol. 990 (2013) 1–35.
[12] V. Kulasingam, E.P. Diamandis, Strategies for discovering novel cancer biomarkers through utilization of emerging technologies, Nat. Clin. Pract. Oncol. 5 (2008) 588–599.
[13] S. Skvortsov, P. Debbage, W.C. Cho, P. Lukas, I. Skvortsova, Putative biomarkers and therapeutic targets associated with radiation resistance, Expert Rev. Proteomics 11 (2014) 207–214.
[14] R.M. Caprioli, T.B. Farmer, J. Gile, Molecular imaging of biological samples: localization of peptides and proteins using MALDI-TOF MS, Anal. Chem. 69 (1997) 4751–4760.
[15] K. Nakayama, T. Inoue, S. Sekiya, N. Terada, Y. Miyazaki, T. Goto, et al., The C-terminal fragment of prostate-specific antigen, a 2331 Da peptide, as a new urinary pathognomonic biomarker candidate for diagnosing prostate cancer, PLoS ONE 9 (2014) e107234.
[16] H.J. Jeong, Y.G. Kim, Y.H. Yang, B.G. Kim, High-throughput quantitative analysis of total N-glycans by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, Anal. Chem. 84 (2012) 3453–3460.
[17] J. Yang, J. Zhu, K. He, L.Y. Zhao, L.Y. Liu, T.S. Song, et al., Proteomic profiling of invasive ductal carcinoma (IDC) using magnetic beads-based serum fractionation and MALDI-TOF MS, J. Clin. Lab. Anal. 4 (2014) 321–327.
[18] Z. Heger, M.A. Rodrigo, S. Krizkova, O. Zitka, M. Beklova, R. Kizek, et al., Identification of estrogen receptor proteins in breast cancer cells using matrix-assisted laser desorption/ionization time of flight mass spectrometry (Review), Oncol. Lett. 7 (2014) 1341–1344.
[19] J. An, C. Tang, N. Wang, Y. Liu, W. Guo, X. Li, et al., [Preliminary study of MALDI-TOF mass spectrometry-based screening of patients with the NSCLC serum-specific peptides], Zhongguo Fei Ai Za Zhi 16 (2013) 233–239.
[20] E. Roman, M.L. Lunde, T. Miron, S. Warnakulasauriya, A.C. Johannessen, E.N. Vasstrand, et al., Analysis of protein expression profile of oral squamous cell carcinoma by MALDI-TOF-MS, Anticancer Res. 33 (2013) 837–845.
[21] A. Padoan, R. Seraglia, D. Basso, P. Fogar, C. Sperti, S. Moz, et al., Usefulness of MALDI-TOF/MS identification of low-MW fragments in sera for the differential diagnosis of pancreatic cancer, Pancreas 42 (2013) 622–632.
[22] N. Kocevar, P. Hudler, R. Komel, The progress of proteomic approaches in searching for cancer biomarkers, N. Biotechnol. 30 (2013) 319–326.
[23] N. Zhong, Y. Cui, X. Zhou, T. Li, J. Han, Identification of prohibitin 1 as a potential prognostic biomarker in human pancreatic carcinoma using modified aqueous two-phase partition system combined with 2D-MALDI-TOF-TOF-MS/MS, Tumour Biol. 36 (2015) 1221–1231.
[24] Y.W. Liang, J. Zheng, X. Li, W. Zheng, T. Chen, Selenadiazole derivatives as potent thioredoxin reductase inhibitors that enhance the radiosensitivity of cancer cells, Eur. J. Med. Chem. 84 (2014) 335–342.
[25] R. Wei, Y. Zhang, L. Shen, W. Jiang, C. Li, M. Zhong, et al., Comparative proteomic and radiobiological analyses in human lung adenocarcinoma cells, Mol. Cell. Biochem. 359 (2012) 151–159.
[26] G. Li, B. Xie, X. Li, Y. Chen, Y. Xu, M. Xu-Welliver, et al., Downregulation of peroxiredoxin-1 by beta-elemene enhances the radiosensitivity of lung adenocarcinoma xenografts, Oncol. Rep. 33 (2015) 1427–1433.
[27] A.S. Allal, T. Kahne, A.K. Reverdin, H. Lippert, W. Schlegel, M.A. Reymond, Radioresistance-related proteins in rectal cancer, Proteomics 4 (2004) 2261–2269.
[28] P.L. Ross, Y.N. Huang, J.N. Marchese, B. Williamson, K. Parker, S. Hattan, et al., Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents, Mol. Cell. Proteomics 3 (2004) 1154–1169.
[29] L.R. Zieske, A perspective on the use of iTRAQ reagent technology for protein complex and profiling studies, J. Exp. Bot. 57 (2006) 1501–1508.
[30] X.Z. Cai, W.Q. Zeng, Y. Xiang, Y. Liu, H.M. Zhang, H. Li, et al., iTRAQ-based quantitative proteomic analysis of nasopharyngeal carcinoma, J. Cell. Biochem. 116 (2015) 1431–1441.
[31] B. Kristjansdottir, K. Levan, K. Partheen, E. Carlsohn, K. Sundfeldt, Potential tumor biomarkers identified in ovarian cyst fluid by quantitative proteomic analysis, iTRAQ, Clin. Proteomics 10 (2013) 4.
[32] I. Rehman, C.A. Evans, A. Glen, S.S. Cross, C.L. Eaton, J. Down, et al., iTRAQ identification of candidate serum biomarkers associated with metastatic progression of human prostate cancer, PLoS ONE 7 (2012) e30885.
[33] F. Abdi, J.F. Quinn, J. Jankovic, M. McIntosh, J.B. Leverenz, E. Peskind, et al., Detection of biomarkers with a multiplex quantitative proteomic platform in cerebrospinal fluid of patients with neurodegenerative disorders, J. Alzheimers Dis. 9 (2006) 293–348.
[34] L. Smith, O. Qutob, M.B. Watson, A.W. Beavis, D. Potts, K.J. Welham, et al., Proteomic identification of putative biomarkers of radiotherapy resistance: a possible role for the 26S proteasome?, Neoplasia 11 (2009) 1194–1207.
[35] Y. Ren, P. Hao, B. Dutta, E.S. Cheow, K.H. Sim, C.S. Gan, et al., Hypoxia modulates A431 cellular pathways association to tumor radioresistance and enhanced migration revealed by comprehensive proteomic and functional studies, Mol. Cell. Proteomics 12 (2013) 485–498.
[36] T.E. Angel, U.K. Aryal, S.M. Hengel, E.S. Baker, R.T. Kelly, E.W. Robinson, et al., Mass spectrometry-based proteomics: existing capabilities and future directions, Chem. Soc. Rev. 41 (2012) 3912–3928.
[37] C. Abdallah, E. Dumas-Gaudot, J. Renaut, K. Sergeant, Gel-based and gel-free quantitative proteomics approaches at a glance, Int. J. Plant Genomics 2012 (2012) 494572.
[38] D. Scumaci, L. Tamme, C.V. Fiumara, G. Pappaianni, A. Concolino, E. Leone, et al., Plasma proteomic profiling in hereditary breast cancer reveals a BRCA1-specific signature: diagnostic and functional implications, PLoS ONE 10 (2015) e0129762.
[39] Z. He, B. Hu, L. Tang, S. Zheng, Y. Sun, Z. Sheng, et al., The overexpression of MRP4 is related to multidrug resistance in osteosarcoma cells, J. Cancer Res. Ther. 11 (2015) 18–23.
[40] Y. Kim, D. Han, H. Min, J. Jin, E.C. Yi, Y. Kim, Comparative proteomic profiling of pancreatic ductal adenocarcinoma cell lines, Mol. Cells 37 (2014) 888–898.
[41] X. Zeng, B.L. Hood, T. Zhao, T.P. Conrads, M. Sun, V. Gopalakrishnan, et al., Lung cancer serum biomarker discovery using label-free liquid chromatography-tandem mass spectrometry, J. Thorac. Oncol. 6 (2011) 725–734.
[42] N. Yang, S. Feng, K. Shedden, X. Xie, Y. Liu, C.J. Rosser, et al., Urinary glycoprotein biomarker discovery for bladder cancer detection using LC/MS-MS and label-free quantification, Clin. Cancer Res. 17 (2011) 3349–3359.
[43] L. Gorchs, T. Hellevik, J.A. Bruun, K.A. Camilio, S. Al-Saad, T.B. Stuge, et al., Cancer-associated fibroblasts from lung tumors maintain their immunosuppressive abilities after high-dose irradiation, Front. Oncol. 5 (2015) 87.
[44] H. Yan, K. Yang, H. Xiao, Y.J. Zou, W.B. Zhang, H.Y. Liu, Over-expression of cofilin-1 and phosphoglycerate kinase 1 in astrocytomas involved in pathogenesis of radioresistance, CNS Neurosci. Ther. 18 (2012) 729–736.
[45] P. Picotti, R. Aebersold, Selected reaction monitoring-based proteomics: workflows, potential, pitfalls and future directions, Nat. Methods 9 (2012) 555–566.
[46] J. Lehtio, L. De Petris, Lung cancer proteomics, clinical and technological considerations, J. Proteomics 73 (2010) 1851–1863.
[47] T.B. Schaaij-Visser, R.H. Brakenhoff, C.R. Leemans, A.J. Heck, M. Slijper, Protein biomarker discovery for head and neck cancer, J. Proteomics 73 (2010) 1790–1803.
[48] L. Guo, Y. Xiao, M. Fan, J.J. Li, Y. Wang, Profiling global kinome signatures of the radioresistant MCF-7/C6 breast cancer cells using MRM-based targeted proteomics, J. Proteome Res. 14 (2015) 193–201.
[49] D.M. Cox, F. Zhong, M. Du, E. Duchoslav, T. Sakuma, J.C. McDermott, Multiple reaction monitoring as a method for identifying protein posttranslational modifications, J. Biomol. Tech. 16 (2005) 83–90.
[50] L. Anderson, C.L. Hunter, Quantitative mass spectrometric multiple reaction monitoring assays for major plasma proteins, Mol. Cell. Proteomics 5 (2006) 573–588.
[51] L.C. Gillet, P. Navarro, S. Tate, H. Rost, N. Selevsek, L. Reiter, et al., Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis, Mol. Cell. Proteomics 11 (2012) O111.016717.
[52] J. Vowinckel, F. Capuano, K. Campbell, M.J. Deery, K.S. Lilley, M. Ralser, The beauty of being (label)-free: sample preparation methods for SWATH-MS and next-generation targeted proteomics, F1000Res. 2 (2013) 272.
[53] Y. Liu, J. Chen, A. Sethi, Q.K. Li, L. Chen, B. Collins, et al., Glycoproteomic analysis of prostate cancer tissues by SWATH mass spectrometry discovers N-acylethanolamine acid amidase and protein tyrosine kinase 7 as signatures for tumor aggressiveness, Mol. Cell. Proteomics 13 (2014) 1753–1768.
[54] F. Zhang, H. Lin, A. Gu, J. Li, L. Liu, T. Yu, et al., SWATH- and iTRAQ-based quantitative proteomic analyses reveal an overexpression and biological relevance of CD109 in advanced NSCLC, J. Proteomics 102 (2014) 125–136.

[55] X.P. Feng, H. Yi, M.Y. Li, X.H. Li, B. Yi, P.F. Zhang, et al., Identification of biomarkers for predicting nasopharyngeal carcinoma response to radiotherapy by proteomics, Cancer Res. 70 (2010) 3450–3462.
[56] B. Zhang, J.Q. Qu, L. Xiao, H. Yi, P.F. Zhang, M.Y. Li, et al., Identification of heat shock protein 27 as a radioresistance-related protein in nasopharyngeal carcinoma cells, J. Cancer Res. Clin. Oncol. 138 (2012) 2117–2125.
[57] P. Wu, H. Zhang, L. Qi, Q. Tang, Y. Tang, Z. Xie, et al., Identification of ERp29 as a biomarker for predicting nasopharyngeal carcinoma response to radiotherapy, Oncol. Rep. 27 (2012) 987–994.
[58] L. Li, S. Huang, X. Zhu, Z. Zhou, Y. Liu, S. Qu, et al., Identification of radioresistance-associated proteins in human nasopharyngeal carcinoma cell lines by proteomic analysis, Cancer Biother. Radiopharm. 28 (2013) 380–384.
[59] T.Y. Lin, J.T. Chang, H.M. Wang, S.H. Chan, C.C. Chiu, C.Y. Lin, et al., Proteomics of the radioresistant phenotype in head-and-neck cancer: Gp96 as a novel prediction marker and sensitizing target for radiotherapy, Int. J. Radiat. Oncol. Biol. Phys. 78 (2010) 246–256.
[60] J.S. Kim, J.W. Chang, H.S. Yun, K.M. Yang, E.H. Hong, D.H. Kim, et al., Chloride intracellular channel 1 identified using proteomic analysis plays an important role in the radiosensitivity of HEp-2 cells via reactive oxygen species production, Proteomics 10 (2010) 2589–2604.
[61] S.Y. Lee, H.R. Park, N.H. Cho, Y.P. Choi, S.Y. Rha, S.W. Park, et al., Identifying genes related to radiation resistance in oral squamous cell carcinoma cell lines, Int. J. Oral Maxillofac. Surg. 42 (2013) 169–176.
[62] T. Wang, D. Tamae, T. LeBon, J.E. Shively, Y. Yen, J.J. Li, The role of peroxiredoxin II in radiation-resistant MCF-7 breast cancer cells, Cancer Res. 65 (2005) 10338–10346.
[63] M.H. Kim, S.Y. Jung, J. Ahn, S.G. Hwang, H.J. Woo, S. An, et al., Quantitative proteomic analysis of single or fractionated radiation-induced proteins in human breast cancer MDA-MB-231 cells, Cell Biosci. 5 (2015) 2.
[64] I. Skvortsova, S. Skvortsov, T. Stasyk, U. Raju, B.A. Popper, B. Schiestl, et al., Intracellular signaling pathways regulating radioresistance of human prostate carcinoma cells, Proteomics 8 (2008) 4521–4533.
[65] W. Huang, X. Ding, B. Li, M. Fan, T. Zhou, H. Sun, et al., Serum biomarkers analyzed by LC-MS/MS as predictors for short outcome of non-small cell lung cancer patients treated with chemoradiotherapy, Neoplasma 60 (2013) 11–18.
[66] S. Marur, A.A. Forastiere, Head and neck cancer: changing epidemiology, diagnosis, and treatment, Mayo Clin. Proc. 83 (2008) 489–501.
[67] M. Parfenov, C.S. Pedamallu, N. Gehlenborg, S.S. Freeman, L. Danilova, C.A. Bristow, et al., Characterization of HPV and host genome interactions in primary head and neck cancers, PNAS 111 (2014) 15544–15549.
[68] J.E. Bauman, L.S. Michel, C.H. Chung, New promising molecular targets in head and neck squamous cell carcinoma, Curr. Opin. Oncol. 24 (2012) 235–242.
[69] S. Skvortsov, C.R. Jimenez, J.C. Knol, P. Eichberger, B. Schiestl, P. Debbage, et al., Radioresistant head and neck squamous cell carcinoma cells: intracellular signaling, putative biomarkers for tumor recurrences and possible therapeutic targets, Radiother. Oncol. 101 (2011) 177–182.
[70] R. Holland, S.H. Veling, M. Mravunac, J.H. Hendriks, Histologic multifocality of Tis, T1-2 breast carcinomas. Implications for clinical trials of breast-conserving surgery, Cancer 56 (1985) 979–990.
[71] L. Smith, O. Qutob, M.B. Watson, A.W. Beavis, D. Potts, K.J. Welham, Proteomic identification of putative biomarkers of radiotherapy resistance: a possible role for the 26S proteasome?, Neoplasia 11 (2009) 1194–1207.
[72] D. Khuntia, C.A. Reddy, A. Mahadevan, E.A. Klein, P.A. Kupelian, Recurrence-free survival rates after external-beam radiotherapy for patients with clinical T1-T3 prostate carcinoma in the prostate-specific antigen era: what should we expect?, Cancer 100 (2004) 1283–1292.
[73] M. Anwar, V. Weinberg, A.J. Chang, I.C. Hsu, M. Roach 3rd, A. Gottschalk, Hypofractionated SBRT versus conventionally fractionated EBRT for prostate cancer: comparison of PSA slope and nadir, Radiat. Oncol. 9 (2014) 42.