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Proteomic Findings in Melanoma

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Abstract

Although the emergence of proteomics as an independent branch of science is fairly recent, within a short period of time it has contributed substantially in various disciplines. The tool of mass spectrometry has become indispensable in the analysis of complex biological samples. Clinical applications of proteomics include detection of predictive and diagnostic markers, understanding mechanism of action of drugs as well as resistance mechanisms against them and assessment of therapeutic efficacy and toxicity of drugs in patients. Here, we have summarized the major contributions of proteomics towards the study of melanoma, which is a deadly variety of skin cancer with a high mortality rate.

Keywords

Proteomics; Melanoma; Biomarkers; Mass spectrometry

Proteomics encompasses large-scale analyses involving the structure or function of proteins. The contribution of proteomics in various fields of science is well-acknowledged. Since the amount, type and activity of proteins synthesized inside a cell is constantly governed by external stimuli, proteomic analyses can provide vital cellular information at any point of time. Here, we have summarized the major findings in melanoma in the past five years that can be attributed to proteomics.

Melanoma is a deadly disease that accounts for a majority (~ 75%) of skin-cancer related deaths. The incidence of melanoma has almost doubled since 1973, and it keeps increasing every year. The advanced stage of the disease is associated with poor prognosis and very low survival rate; stage IV melanoma typically has a 5 year survival rate of approximately 15–20%. Despite of the increasing incidence of melanoma cases, the survival rate of melanoma patients has improved over the years due to improved diagnosis, and availability of better treatment options. The field of proteomics has contributed significantly in the development of effective diagnostic and prognostic tools available to clinicians currently.

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A major role played by proteomics in melanoma has been identification of potential biomarkers for diagnostic purposes. Efficient, sensitive and specific biomarkers are key to early diagnosis and initiation of effective treatment and tremendous progress in the field of proteomics in the past few years have resulted in the discovery of several putative biomarkers through comprehensive proteomic analysis of cell lines, tissues and serum [1]. Proteomics is an alternative tool for biomarker discovery compared to genomics, since it does not call for constant access to fresh tissue unlike genomic profiling [2]. Although it is possible to perform quantitative gene profiling using formalin-fixed-paraffin-embedded (FFPE) tissues, but the RNA derived from such archived tissues are often degraded and fragmented which sometimes results in "FFPE bias" [3]. Recent work by Kawahara et al. [4], showed the potential use of discovery-based proteomics data obtained from the secretion of melanoma cell lines along with other human cell lines. They used a MS-based approach followed by clustering techniques and bioinformatics to determine proteins that were differentially expressed in the melanoma cell lines (A2058 and SK-MEL-28) compared to noncancerous cell lines (HaCaT and HEK293). The differentially identified proteins were later validated by other approaches like immunoblotting and tissue microarrays. Using this approach they were able to identify 271 potential biomarkers for melanoma.

Over the years, proteomics has emerged as a vital tool for the identification of active molecular pathways and unveiling mechanisms of pathogenesis as well as drug action and resistance. Byrum et al. [5] used quantitative proteomics on formalin-fixed paraffin-embedded human melanoma tissues to identify molecular pathways that were aberrant in melanoma. They were able to identify 171 proteins that were differentially expressed in the three types of tissues - benign nevi, primary melanoma, and metastatic melanoma, many of which constitute molecular pathways associated with apoptosis, tumor cell proliferation and cell motility. These provided mechanistic insights into pathogenesis associated with advanced melanoma. In 2014, Rebecca et al. [6] used liquid chromatography-multiple reaction monitoring mass spectrometry (LC-MRM) to study the molecular mechanisms of responses of melanoma cells to MEK and HSP90 inhibitors. Another study used quantitative protein profiling by tandem mass spectrometry for comprehensive proteomic analysis of responders and non-responders to Dacarbazine (DTIC) or temozolomide (TMZ) chemotherapy [7]. In this study, the group was able to detect S100A13 as the protein responsible for resistance to chemotherapy in the non-responders. Singh et al. [8] utilized a gel free quantitative proteomics approach to identify targets of the histone deacetylase SIRT1, which is upregulated in melanoma. Upon treatment with a SIRT1 inhibitor, they identified 1091 proteins of which 20 were differentially expressed in the treatment group, including the BUB family proteins (BUB3, BUB1 and BUBR1). Using proteomics approaches, they were able to conclude that BUB family proteins are downstream targets of SIRT1. Cholewa et al. [9] used label-free comparative proteomics analysis with nano-LC-MS/MS technology to determine the cause of failure of Polo-like kinase 1 (Plk1) inhibitors as cancer therapeutics. When they performed a large-scale comprehensive analysis of proteins in BRAF mutant melanoma cells treated with a Plk1-specific inhibitor, they detected down-regulation of several proteins including metabolic proteins and multiple proteosomal subunits and up-regulation of proteins like hnRNPC all of which provided mechanistic insights into the function of Plk-1 in cancer. In 2015, Lai et al. [10] used a

temporal quantitative proteomics approach, iTRAQ 2D-LC-MS/MS, to reveal insight into the mechanism of cytotoxicity of the drug panduratin A (PA) in A375 melanoma cells. They found that proteins associated with mitochondrial oxidative phosphorylation, ER stress pathway, and apoptosis were down-regulated in cells treated with PA indicating prolonged ER stress as the primary cause of apoptosis. Hence, it can be concluded that the tool of proteomics coupled with the recent advancements in the field of bioinformatics have proved to be tremendously useful in understanding the complexities of drug action and resistance, which contributes significantly to designing effective treatment regimens.

Often more than one proteomic approach is used to solve a particular problem. In 2013, Gholami et al. [11] investigated protein and kinase expression in the model system NCI-60 cell line using a combination of three proteomic approaches - proteomic profiling, kinomic profiling and deep proteomics. Overall, 10,350 proteins (including 375 protein kinases and a core cancer proteome of 5,578 proteins) were quantified across all nine tissue types (including melanoma cell lines). Bioinformatic evaluation identified hundreds of potential biomarkers along with potential protein markers for drug sensitivity and resistance. Another such study utilizing more than one proteomic approaches was performed recently by Paulitschke et al. [12] who used shotgun analysis, pressure cycling technology, and selected reaction monitoring to investigate the mechanism of resistance against BRAF inhibitors in melanoma patients. Using these techniques they showed that BRAFi resistance is caused chiefly by epithelial-mesenchymal transformation that occurs when melanoma cells turn invasive as a result of treatment using BRAFi. Some of the major contributions of proteomics in the field of melanoma are summarized in Table 1.

Although complex analyses involved in large-scale proteomics poses challenges, the tool of proteomics has proved to be indispensable in various fields of cancer including melanoma for identification of novel and effective diagnostic and prognostic markers. Furthermore, the growing field of proteomics is increasingly being used for effective drug design, in addition to understanding drug action and mechanism of resistance. It can be expected that with the recent progress in proteomics, it will soon be possible to customize medications personalized for each individual to guarantee maximum effectiveness with least toxicity.

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Table 1

Major contributions of proteomics in melanoma.

Author	Year	Proteomics technique used	Major finding
Chen et al. [13]	2011	2 D gel electrophoresis and matrix-assisted laser desorption ionization-time-of-flight/time-of-flight (MALDI/TOF)	Hypoxia-inducible promoter - adhE promoter was screened from the anaerobically regulated proteins of Salmonella
Xiao et al. [14]	2012	Differential proteomics using 2-D DIGE (two-dimensional difference in gel electrophoresis) followed my mass spectrometry	Identified exosomal proteins that were differentially expressed in metastatic melanoma compared to melanocytes
Hashimoto et al. [15]	2012	Sucrose density gradient ultracentrifugation of Triton X-100 extracts or enzyme-mediated activation of radical sources (EMARS) reaction followed by mass spectrometry	EMARS reaction could be used to identify ganglioside-interacting membrane proteins
Hughes et al. [16]	2012	SILAC MS-based proteomics screen	Analysis of extracellular matrix revealed over 80 extracellular proteins that stimulated pluripotent stem cells
Ye et al. [17]	2013	Quantitative shotgun proteomics	Differential (18)O/(16)O stable isotopic labeling was used to identify hypoxia-induced protein markers in malignant melanoma
Steunou et al. [18]	2013	Affinity purification followed by mass spectrometry and label-free quantification	Identified proteins interacting with hypoxia-inducible factor 2α (HIF2α) that contributed to melanoma progression
Paulitschke et al. [19]	2013	Mass spectrometry-based proteome profiling of cisplatinresistant *vs.* sensitive cells	Lysosomal, survival and cell adherence related proteins of cisplatin resistant cells were higher compared to sensitive cells.
Myers et al. [20]	2013	Targeted mass spectrometry based on an SRM peptide quantification method	novel biomarker predictor for preeclampsia identified
Li et al. [21]	2013	Comparative proteomic analysis using Two-dimensional gel electrophoresis	Proteins associated with mitochondrial dysfunction and apoptosis were differentially expressed in A375 melanoma cells treated with sinulariolide.
James et al. [22]	2013	Phosphoproteomics and mass spectrometry	Protein kinase N1 forms complex with WNT3A receptor and block Wnt/β-catenin signaling
Byrum et al. [5]	2013	Comparative proteomics analysis using nanoflow LC-MS/MS	Analysis of 61 FFPE human tissues including benign nevi, primary melanoma, and metastatic melanoma identified 171 significantly varying proteins associated with proliferation, motility, and apoptosis.
Tang et al. [23]	2014	Proteomic profiling performed by MS/MS	Anti-cancer effect of Phylllanthus is due to inhibition of MAPK/ERK, hypoxia, Myc/Max and NFκB pathways
Xiao et al. [24]	2014	multiple-reaction monitoring (MRM) used to profile kinase expression in melanoma cell lines	Cancer progression is associated with major kinome reprogramming
Kotobuki et al. [25]	2014	Isobaric tags for relative and absolute quantitation (iTRAQ)	Over-expression of extracellular matrix protein, periostin (POSTN), in metastatic melanoma compared to normal skin
Qendro et al. [26]	2014	Tandem mass spectrometry	Identification of nestin and vimentin as potential biomarkers
Smit et al. [27]	2014	(phospho)proteomic	ROCK1 inhibitor sensitizes melanoma cells to BRAF inhibitors
Liu et al. [28]	2014	2-DE based comparative proteomics	Gallic acid induced apoptosis is coupled with glycolysis in B16F10
Strickler et al. [29]	2014	Tandem mass spectrometry	Identified proteins associated with pathogenesis, for potential diagnostic purpose
Kraya et al. [30]	2015	Comparative quantitative proteomics using secretome of 3 D cell culture	Candidate autophagy biomarkers were identified

Author	Year	Proteomics technique used	Major finding
Welinder et al. [31]	2015	Deep mining proteomics	A metastatic melanoma protein sequence database was built having 5000 unique proteins that can be potential biomarkers
Yu et al. [32]	2015	Targeted quantitative proteomics (Selected reaction monitoring)	Identified novel endogenous substrates of Human Kallikrein 7 (serine protease)
Raaijmakers et al. [33]	2015	Comparative quantitative proteomics on cell lines derived from patients	PhosphoPath – an app designed for visualization and analysis of phosphoproteome data
Hao et al. [34] (34)	2015	S *in vivo/vitro* labelling analysis for dynamic proteomics (SiLAD)	Proliferation inhibited by miR-137 in melanoma cells by reduced p21- activated kinase 2 (PAK2) expression rate
Makowski et al. [35]	2016	Proteome-wide survey of transcription factors	ELF1 binds to somatic mutations of oncogenic TERT promoter
Sengupta et al. [36]	2016	Label-free precursor ion intensity approach for bottom-up analysis of histone PTMs	EZH2 promotes H3K27me3-mediated silencing of RUNX3 and E-cadherin tumor suppressors in melanoma