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MINI-REVIEW

Targeting proteomics to investigate metastasis-associated mitochondrial proteins

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Abstract Mitochondria are essential organelles in eukaryotic cells and are responsible for regulating energy metabolism, ROS production, and cell survival. Recently, various cellular pathogeneses, including tumorigenesis and metastasis, have been reported to be associated with mitochondrial homeostasis. Consequently, exploiting the correlation between dysfunctional mitochondria and tumor progression has been implicated in the understanding of tumorigenesis, tumor metastasis, and chemoresistance, along with novel strategies to develop cancer therapeutics. To comprehensively understand the role of the mitochondria in cancer metastasis, it is necessary to resolve thousands of mitochondrial proteins and their post-translational modifications with high-throughput global assessments. We introduce mitochondrial proteomic strategies in this review and a discussion on their recent findings related to cancer metastasis. Additionally, the mitochondrial respiratory chain is believed to be a major site for ROS production, and elevated ROS is likely a key source to trigger dysfunctional mitochondria and impaired mitochondrial metabolism that subsequently contribute to the development of cancer progression. Equipment-based metabolomic analysis now allows the monitoring of disease progression and diagnosis. These newly emerging techniques, including proteomics, redox-proteomics, and metabolomics, are described in this review.

Keywords Proteomics · Mitochondria · Metastasis

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Abbreviations

2-DE	two-dimensional gel electrophoresis
CCB	colloidal coomassie blue
dd H2O	double deionized water
DIGE	differential gel electrophoresis
MALDI-TOF MS	matrix assisted laser desorption ionization-time of flight mass spectrometry

The relationship between mitochondrial defects and cancer progression

Whereas clinical signs and symptoms of cancer vary, the principal features of most cancerous cells include metabolic imbalances, generation of reactive oxygen species (ROS), and resistance to programmed cell death (apoptosis). Tumorigenesis relies heavily on enhanced uptake and consumption of glucose to meet metabolic demands, even in the presence of abundant oxygen (Warburg 1926). Warburg suggested that aerobic glycolysis in malignant cells is commonly caused by impaired mitochondrial energy metabolism. Several mechanisms have been proposed to clarify this phenomenon, including the upregulation of rate-limiting processes of glycolysis through the activation of the Akt pathway and the expression of Ras or Src oncogenes (Pelicano et al. 2006), the alteration to hypoxia induced from mitochondrial respiration by oxidative phosphorylation (OXPHOS) to glycolysis (Robey and Hay 2006), and the accumulation of mutations in specific mitochondrial proteins such as succinate dehydrogenases, fumarate hydratase, and isocitrate dehydrogenases, which may in turn activate the hypoxia-inducible factor (HIF) and create a pesudohypoxic response that leads to aerobic glycolysis and cancer development (King et al. 2006). Additionally, glycolytic ATP production is linked to local acidification by lactate production and acidification of the tumor microenvironment, which acts as an immune escape that may facilitate

tumor cell aggressiveness and tumor invasion (Calcinotto et al. 2012; Simonnet et al. 2002). Mitochondrial respiration also produces ROS, which influences many physiological processes and is detrimental to the cell if overproduced. Investigations have reported that ROS can non-specifically and rapidly oxidize proteins, lipids, and DNA (Andreyev et al. 2005); therefore, ROS represents a major oxidative stress to lethal cell injury. Because mitochondria are the major locus for oxygen-radical production, the electron transport chain is associated with the mitochondrial inner membrane, and mitochondrial DNA is more susceptible to ROS than nuclear DNA because it lacks introns and protective histones. Elevated ROS is likely a key trigger of dysfunctional mitochondrial respiration and mitochondrial genomic instability observed in cancer initiation and progression (Gogvadze et al. 2008). Because tumor cells actively generate higher levels of ROS compared to normal cells, they are more sensitized to death by ROS-producing agents. Many commonly used cytotoxic anticancer drugs, such as cisplatin, doxorubicin, and ionizing radiation, are designed to promote ROS accumulation and act as signal mediators for exerting their apoptotic action through ROS-mediated mitochondrial damage in cancer cells (Fulda et al. 2010; Gogvadze et al. 2009). Although mitochondria are often referred to as the powerhouse of the cell, its functions are not restricted to ATP production and ROS generation. Mitochondria have attracted attention in cancer research because they are an important control center regulating the intrinsic apoptotic pathway and genetic defects in apoptotic pathways that directly promote cancer cell survival. Furthermore, crosstalk occurs between the extrinsic (by death receptors) and intrinsic (by mitochondria) apoptotic pathways. The intrinsic apoptotic pathway is first characterized by permeabilization of the mitochondrial membrane, which is considered a "point of no return" for the apoptotic process and tightly regulated by Bcl-2 family proteins. Once triggered, the mitochondrial outer membrane leaks, resulting in the release of cytochrome *c* and apoptosis-inducing factors into the cytoplasm. Cytochrome *c* then forms a multiprotein complex known as the apoptosome-containing adaptor Apaf-1 and caspase 9 to initiate activation of the caspase cascade and execute cell apoptosis (Green 1998). Apoptosis is of crucial importance to halt the replication process of damaged and aging cell populations as a homeostatic mechanism. Because the majority of tumor cells are resistant to apoptosis, it is important to know whether mitochondria-associated perturbations and bioenergetics that lead to apoptotic resistance in malignant cells are different from the mitochondria-associated perturbations and bioenergetics of the mitochondria in non-malignant cells.

During multistep oncogenesis, metastasis is a complex process that causes 90 % of human cancer deaths in the Weigelt model (Weigelt et al. 2005). However, apoptosis occurs early in metastatic inefficiency, and resistance to apoptosis is increased during metastasis. Suppression of crucial apoptotic modulators provides a multistep barrier to metastasis (Mehlen and Puisieux 2006). Consequently, exploiting mitochondrial apoptosis has been implicated in the search for novel anticancer drugs against tumorigenesis, tumor metastasis, and chemoresistance. For example, cells deficient in cytochrome *c* correspond to the resistance of UV or anticancer drug-mediated apoptosis (Li et al. 2000). Mutations in Bax, a pro-apoptotic protein belonging to the Bcl-2 family, have been documented as an anti-apoptosis mechanism against UV, bleomycin, cisplatin, staurosporine, and VP-16 (Wang et al. 2001; Zhang et al. 2000). Reduced mitochondrial ROS, a byproduct of OXPHOS, may provide an antioxidant-buffering system in the Warburg effect, whereas promoting mitochondrial ROS production may enhance the susceptibility of tumor cells to undergo apoptosis (Levine and Puzio-Kuter 2010). Therefore, targeting the mitochondria appears to be a promising therapeutic strategy to explore the interplay between apoptotic regulators and altered mitochondrial metabolism for the induction of cellular energy metabolism, the promotion of ROS production, and the activation of apoptosis in tumor cells. Therefore, the coordinated attack aimed at killing cancer cells and suppressing tumor metastasis should be based on the concerted modulation of mitochondrial stability and signaling. However, it is essential to selectively and efficiently regulate complex mitochondrial alternations in malignantly transformed cells. Faster, more sensitive, and more specific biomarkers are needed to detect and treat cancer. Recent advances in methodology and the application of proteomics, redox-proteomics, and metabolomics have advanced in identifying comprehensive cancer biomarkers and developing novel cancer therapeutics cost-effectively.

Current techniques for investigating comparative mitochondrial proteome

Mitochondrial proteins are encoded by the nuclear genome and the mitochondrial genome, with an estimated 1,000 proteins existing in the mitochondria (Verma et al. 2003). Because mitochondria regulate physiological processes, including cell proliferation, cellular metabolism, and cell death, alterations of the mitochondrial proteome reflect the beginnings of human disease. Recent advances in proteomic techniques allow comparisons of the differential mitochondrial proteomes between the normal state and disease state of mitochondria. Reports have documented strategies to investigate mitochondrial proteomes, including mitochondria isolation and proteomic analysis, to emphasize its biochemical versatility and importance (Calvo and Mootha 2010; Da Cruz et al. 2005; Herrmann and Herrmann

2012). Techniques that are frequently used to identify the differential proteomes include two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS). The 2-DE relies on the separation of proteins in the first dimension according to the charge of the proteins, and in the second dimension according to the relative molecular weights of the proteins. This allows high-resolution protein separation and detection to provide pure samples for subsequent MS analysis and for highly sensitive analysis of differential protein expression profiles. However, 2-DE-based proteomic studies are limited because low abundant proteins cannot be visualized and because strongly basic or hydrophobic proteins such as membrane proteins have poor resolution. A significant improvement was realized by the introduction of the two-dimensional difference gel electrophoresis (2D-DIGE) technique, in which different protein samples can be co-monitored on the same 2-DE using differential fluorescent dye (Cy2-, Cy3- and Cy5-dye) labeling. This method alleviates gel-to-gel variation, closes the gap across gels relative to the resolved proteins through a fluorescently labeled internal standard, offers a broader dynamic range of detection, a greater reproducibility, and a higher sensitivity than conventional 2-DE (Lai et al. 2010; Timms and Cramer 2008; Wu et al. 2012). Additionally, MS-based proteomics allow the determination of the masses of peptides/proteins with high accuracy. Two major approaches used in the identification of peptides and proteins are matrix-assisted laser-desorption/ionization mass spectrometry (MALDI-TOF MS) (Karas et al. 2000; Karas and Hillenkamp 1988) and electrospray ionization mass spectrometry (ESI-MS) (Fenn et al. 1989; Whitehouse et al. 1985), which depend on peptide mass fingerprinting and peptide fragmentation, respectively, for protein identification. The combination of liquid chromatography (LC) and ESI-MS facilitates the quantification across different proteomes through in vitro and in vivo labeling strategies. Quantitative proteomic analysis includes isotope-coded affinity tags (ICATs) (Dunkley et al. 2004; Gygi et al. 1999), isobaric tags for relative and absolute quantitation (iTRAQ) (DeSouza et al. 2005; Evans et al. 2012), and stable isotope labeling with amino acids in cell culture (SILAC) for the accurate quantitative determination of protein amounts (Blagoev et al. 2003; Pimienta et al. 2009).

Mitochondrial proteomic analysis of metastatic proteins

Defects in the mitochondria have been linked to life-threatening diseases such as cancer metastasis. Hundreds of mitochondrial DNA mutations have been examined in metastatic cancer cells (Goswami 2009; Tu et al. 2011). However, mitochondrial DNA screening is limited to genes encoded by mitochondrial DNA, although larger populations of mitochondrial proteins are encoded by nuclear genes. Accordingly, to extend the detection of defected mitochondrial functions associated with cancer cell metastasis, it is crucial to complement mitochondrial DNA research with proteomic analysis. However, few mitochondrial proteomic analyses have been performed to analyze the differentially expressed mitochondrial proteomes from normal cells to metastasis. These studies are being conducted to investigate breast cancer and oral cancer.

In breast cancer research, Chen et al. enriched mitochondrial proteins from 3 breast cells, MCF10A, MCF7, and MDA-MB-231, corresponding to normal luminal epithelial cells, noninvasive breast cancer cells derived from the luminal duct, and invasive breast cancer cells derived from the same tissues, respectively, followed by quantitatively identified potential mitochondrial transformation markers of breast cancer by 2D-DIGE and MALDI-TOF mass spectrometry. The mitochondrial proteomic results showed that more than 1,500 protein features were resolved and 125 differentially expressed proteins were identified by their peptide mass fingerprintings, 18 of which identified mitochondrial proteins that have not been reported in breast cancer-related metastatic research. The authors further validated the identified mitochondrial metastatic markers, MnSOD and SCaMC-1, by both immunoblot and immunofluorescent analysis, with the results coinciding with the proteomic analysis. The authors also examined the level of the newly identified mitochondrial protein SCaMC-1 in clinical plasma specimens and showed that SCaMC-1 significantly increased in both metastatic and non-metastatic breast cancer patients. Thus, SCaMC-1 is potentially a cancer progression biomarker for breast cancer diagnosis. The authors also demonstrated that prohibitin, a major mitochondria-located protein, was dominantly distributed in the mitochondria of the MCF-10A cells, but was confined within the nucleus in noninvasive breast cancer cells (MCF-7 and MDA-MB-453) and in invasive breast cancer cells (MDA-MB-231, MDA-MB-361, MDA-MB-435 and SKBR3), implying that prohibitin was differentially distributed between normal breast cells and breast cancer cells, and that the subcellular locations of this protein may account for tumorigenesis and metastasis (Chen et al. 2011).

Chen et al. demonstrated that circulating BCM2 parent cells from blood of a stage IV breast cancer patient with widely spread metastasis were established for metastatic research. BCM2 parent cells were injected into female severe combined immunodeficient (SCID) mice, and metastatic tumor cells (BCM2 BrainG1) from a brain lesion were harvested 6 weeks post-injection. After proteomic analysis with multidimensional chromatography and tandem MS, the proteome profiles indicated increased expression of metabolic proteins involving glycolysis, the citric acid cycle, oxidative phosphorylation, beta-oxidation, the glutathione

system, and the pentose pathway, implying that metastatic breast cancer cells gain energy mainly through aerobic glucose oxidation from an enhanced oxidative metabolism. This suggests that metastatic cancer cells undergo a metabolic switch that enhances mitochondrial respiration pathways for energy generation and indirectly promotes metastatic cancer cell survival and proliferation in the target microenvironment. This corresponds to the Warburg effect and offers a therapeutic target for inhibiting breast cancer metastasis (Chen et al. 2007).

Herrmann et al. indicated that the subunit levels of the mitochondrial protein, cytochrome *c* oxidase, were correlated to the progression of prostate cancer from normal tissue through premalignant lesions to aggressive prostate cancer (Herrmann et al. 2003). Mitochondrial cytochrome *c* oxidase contains 13 subunits, in which subunits I, II, and III are encoded by mitochondrial DNA, whereas the other 10 subunits are encoded by nuclear genes. Laser capture microdisection, immunoblotting analysis, and reverse phase protein lysate array techniques quantitatively determined the ratios between subunits I/II of cytochrome *c* oxidase and subunits IV/V/VI of cytochrome *c* oxidase (Paweletz et al. 2001). The results indicated that the increased ratios of nucleus-encoded subunits to mitochondria-encoded subunits in cytochrome *c* oxidase during the progression of prostate cancer. Similar observations have been found in ovary cancer, colon cancer, breast cancer, and esophageal cancer, implying that the ratio changes are correlated to tumorigenesis and metastasis and are not unique to prostate cancer. Therefore, these studies implicate a universal property of tumorigenesis and metastasis, which causes an imbalance in cytochrome *c* oxidase subunit levels in cancer cells, but not in normal cells. Such a microenvironment modification may induce cells converse from normal to primary tumor and subsequent metastasis that may provide an opportunity for the development of new agents for cancer therapy.

Future directions of omics analysis to study mitochondria and cancer metastasis

Electron transport through the mitochondrial respiratory chain is efficient, and the vast majority of oxygen is normally consumed. However, 1–2 % of electrons are leaked to generate O_2^- in reactions mediated by coenzyme Q and ubiquinone and its complexes. Therefore, mitochondria are believed to be a major site of ROS production in vivo (Boveris and Chance 1973). Metastatic cancer cells undergo an enhanced mitochondrial respiration for energy generation, implying that excess ROS is produced and oxidizes mitochondrial DNA, lipid, and proteins, altering mitochondrial stability and initiating tumorigenesis. Recent studies have demonstrated the relationship between mitochondria-generated ROS and cancer metastasis. Mitochondrial ROS-induced mitochondrial DNA damage increases cancer cell metastasis in a mouse tail-vein metastasis model. Additionally, mutated mitochondrial DNA continuously activates the PI3 kinase pathway that contributes to cancer metastasis (Kulawiec et al. 2009). Goh et al. demonstrated that targeting the overexpression of catalase within the mitochondria of cancer cells significantly suppresses ROS-dependent tumor progression and metastasis, revealing the crosstalk between mitochondrial ROS and cancer cell metastasis (Goh et al. 2011). To understand the ROS-affected mitochondrial proteins and their relationship with cancer metastasis, strategies that can efficiently monitor ROS-modulated proteins should be established.

Several functional groups are potential cellular targets of ROS. One of these targets is the free thiol group (RSH) of cysteine, which is a potent nucleophilic moiety and can undergo numerous redox-induced modifications. Oxidative modifications of RSH groups other than disulphide formation, including the formations of sulfenic acid (RSOH), sulfinic acid (RSO2H), and sulfonic acid (RSO3H), depend on the oxidative capacity of the oxidant (Schafer and Buettner 2001). Oxidation of RSH groups to sulfinic and sulfonic acids is irreversible under physiologic conditions, whereas sulfenic acids are thought to exist only as transient intermediates. Chan et al. reported redox-DIGE analysis to monitor thiol reactivity alteration through the whole proteome. This strategy is based on a pair of iodoacetylated derivatives of fluorescent cyanine dyes, termed ICy3 and ICy5, which target the reduced thiol group of cysteine residues, but are unable to label oxidized forms of the thiol group. Therefore, these dyes are used as probes to monitor changes in cysteine thiol oxidation in response to cellular oxidative stress in quantitative proteomic analysis (Chan et al. 2005; Chan et al. 2006; Chan et al. 2012; Chou et al. 2012).

Metabolomics is an emerging high-throughput global metabolite field of study and has recently demonstrated its role in disease progression and diagnosis (Nagrath et al. 2011). The core techniques used for metabolomic analysis include NMR, gas chromatography (LC)-linked mass spectrometry, and high-performance liquid chromatography (HPLC)-linked mass spectrometry (Weckwerth 2010; Zhang et al. 2012). Recent mass spectrometry advances have led to the development of orbitrap mass spectrometry and Fourier-transformation-ion cyclotron resonance mass spectrometry (Han et al. 2008; Koulman et al. 2009). This tracer equipment allows a fundamental understanding of a disease-associated metabolic atlas.

Recent applications of metabolomic analysis on mitochondrial disorders have demonstrated elevated levels of lactate, creatine, uridine, and alanine in both an in vitro

mitochondrial disease model and the plasmas of respiratory chain disease patients (Shaham et al. 2010). Seifert et al. used mitochondrial metabolomics to globally map the metabolite signatures of long chain fatty acid oxidation in the mitochondria and completed the identification of metabolites linked to fat metabolism and insulin signaling in muscle (Seifert et al. 2010). These studies imply that the application of metabolomic analysis can monitor disease-specific metabolite alterations and provide more realistic insights to improve disease identification and prognosis. Although there is no report directly using the metabolomic technique to study the relationship between the mitochondria and metastasis, further applications of metabolomics may potentially be used to examine the fundamental mechanisms of cancer progression and metastasis.

Concluding remarks

Although omics analysis is a burgeoning field, the existing mitochondrial proteomic analysis in the examination of the correlation between the mitochondria and cancer metastasis has provided limited protein candidates for cancer metastatic research. Few mitochondrial studies have been applied in metastatic study, and more proteomic analyses are expected to be conducted to contribute to this research field. More advanced techniques may be introduced to increase the possibility of the identification of low abundant mitochondrial proteins associated with cell metastasis. Mitochondrial proteins with post-translational modifications (e.g., redox-modification of cysteines) may be monitored because the importance of these modified proteins correlates with mitochondrial functions, and subsequent cancer progression has drawn widespread attention. Metabolomics is another emerging field that may enhance the development of the discovery of cancer biomarkers in the mitochondria. These studies may help understand the effects of existing therapeutics and contribute to new metastasis-associated drug screening.

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References

Andreyev AY et al (2005) Mitochondrial metabolism of reactive oxygen species. Biochemistry (Mosc) 70:200–214

Blagoev B et al (2003) A proteomics strategy to elucidate functional protein-protein interactions applied to EGF signaling. Nat Biotechnol 21:315–318

Boveris A, Chance B (1973) The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. Biochem J 134:707–716

Calcinotto A et al. (2012) Modulation of Microenvironment Acidity Reverses Anergy in Human and Murine Tumor-Infiltrating T Lymphocytes. Cancer Res 72:2746–2756

Calvo SE, Mootha VK (2010) The mitochondrial proteome and human disease. Annu Rev Genomics Hum Genet 11:25–44

Chan HL et al (2005) Proteomic analysis of redox- and ErbB2-dependent changes in mammary luminal epithelial cells using cysteine- and lysine-labelling two-dimensional difference gel electrophoresis. Proteomics 5:2908–2926

Chan HL et al (2006) Proteomic analysis of UVC irradiation-induced damage of plasma proteins: serum amyloid P component as a major target of photolysis. FEBS Lett 580:3229–3236

Chan HL et al (2012) Proteomic analysis of redox-dependent changes using cysteine-labeling 2D DIGE. Methods Mol Biol 854:113–128

Chen EI et al (2007) Adaptation of energy metabolism in breast cancer brain metastases. Cancer Res 67:1472–1486

Chen YW et al (2011) Mitochondrial proteomics analysis of tumorigenic and metastatic breast cancer markers. Funct Integr Genomics 11:225–239

Chou HC et al (2012) Proteomic and redox-proteomic analysis of berberine-induced cytotoxicity in breast cancer cells. J Proteomics 75:3158–3176

Da Cruz S et al (2005) Building the mitochondrial proteome. Exp Rev Proteomics 2:541–551

DeSouza L et al (2005) Search for cancer markers from endometrial tissues using differentially labeled tags iTRAQ and cICAT with multidimensional liquid chromatography and tandem mass spectrometry. J Proteome Res 4:377–386

Dunkley TP et al (2004) The use of isotope-coded affinity tags (ICAT) to study organelle proteomes in Arabidopsis thaliana. Biochem Soc Trans 32:520–523

Evans C et al. (2012) An insight into iTRAQ: where do we stand now? Anal Bioanal Chem. doi:10.1007/s00216-012-5918-6

Fenn JB et al (1989) Electrospray ionization for mass spectrometry of large biomolecules. Science 246:64–71

Fulda S et al (2010) Targeting mitochondria for cancer therapy. Nat Rev Drug Discov 9:447–464

Gogvadze V et al (2008) Mitochondria in cancer cells: what is so special about them? Trends Cell Biol 18:165–173

Gogvadze V et al (2009) Mitochondria as targets for cancer chemotherapy. Semin Cancer Biol 19:57–66

Goh J et al (2011) Mitochondrial targeted catalase suppresses invasive breast cancer in mice. BMC Cancer 11:191

Goswami PC (2009) Mutant mitochondria and cancer cell metastasis: quest for a mechanism. Cancer Biol Ther 8:1386–1388

Green DR (1998) Apoptotic pathways: the roads to ruin. Cell 94:695–698

Gygi SP et al (1999) Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. Nat Biotechnol 17:994–999

Han J et al (2008) Towards high-throughput metabolomics using ultrahigh-field Fourier transform ion cyclotron resonance mass spectrometry. Metabolomics 4:128–140

Herrmann PC, Herrmann EC (2012) Mitochondrial proteome: toward the detection and profiling of disease associated alterations. Methods Mol Biol 823:265–277

Herrmann PC et al (2003) Mitochondrial proteome: altered cytochrome c oxidase subunit levels in prostate cancer. Proteomics 3:1801–1810

Karas M, Hillenkamp F (1988) Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal Chem 60:2299–2301

Karas M et al (2000) Ionization in matrix-assisted laser desorption/ionization: singly charged molecular ions are the lucky survivors. J Mass Spectrom 35:1–12

King A et al (2006) Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. Oncogene 25:4675–4682

Koulman A et al (2009) High-resolution extracted ion chromatography, a new tool for metabolomics and lipidomics using a second-generation orbitrap mass spectrometer. Rapid Commun Mass Spectrom 23:1411–1418

Kulawiec M et al (2009) Cancer cell mitochondria confer apoptosis resistance and promote metastasis. Cancer Biol Ther 8:1378–1385

Lai TC et al (2010) Secretomic and Proteomic Analysis of Potential Breast Cancer Markers by Two-Dimensional Differential Gel Electrophoresis. J Proteome Res 9:1302–1322

Levine AJ, Puzio-Kuter AM (2010) The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. Science 330:1340–1344

Li K et al (2000) Cytochrome c deficiency causes embryonic lethality and attenuates stress-induced apoptosis. Cell 101:389–399

Mehlen P, Puisieux A (2006) Metastasis: a question of life or death. Nat Rev Cancer 6:449–458

Nagrath D et al (2011) Metabolomics for mitochondrial and cancer studies. Biochim Biophys Acta 1807:650–663

Paweletz CP et al (2001) Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. Oncogene 20:1981–1989

Pelicano H et al (2006) Glycolysis inhibition for anticancer treatment. Oncogene 25:4633–4646

Pimienta G et al (2009) SILAC for global phosphoproteomic analysis. Methods Mol Biol 527:107–116, x

Robey RB, Hay N (2006) Mitochondrial hexokinases, novel mediators of the antiapoptotic effects of growth factors and Akt. Oncogene 25:4683–4696

Schafer FQ, Buettner GR (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 30:1191–1212

Seifert EL et al (2010) Long-chain fatty acid combustion rate is associated with unique metabolite profiles in skeletal muscle mitochondria. PLoS One 5:e9834

Shaham O et al (2010) A plasma signature of human mitochondrial disease revealed through metabolic profiling of spent media from cultured muscle cells. Proc Natl Acad Sci U S A 107:1571–1575

Simonnet H et al (2002) Low mitochondrial respiratory chain content correlates with tumor aggressiveness in renal cell carcinoma. Carcinogenesis 23:759–768

Timms JF, Cramer R (2008) Difference gel electrophoresis. Proteomics 8:4886–4897

Tu YF et al (2011) Mitochondria of highly metastatic breast cancer cell line MDA-MB-231 exhibits increased autophagic properties. Biochim Biophys Acta 1807:1125–1132

Verma M et al (2003) Proteomic analysis of cancer-cell mitochondria. Nat Rev Cancer 3:789–795

Wang GQ et al (2001) A role for mitochondrial Bak in apoptotic response to anticancer drugs. J Biol Chem 276:34307–34317

Warburg OU (1926) Translated: The metabolism of tumors. London: Arnold Constable. Springer, Berlin

Weckwerth W (2010) Metabolomics: an integral technique in systems biology. Bioanalysis 2:829–836

Weigelt B et al (2005) Breast cancer metastasis: markers and models. Nat Rev Cancer 5:591–602

Whitehouse CM et al (1985) Electrospray interface for liquid chromatographs and mass spectrometers. Anal Chem 57:675–679

Wu CL et al. (2012) Proteomic analysis of UVB-induced protein expression- and redox-dependent changes in skin fibroblasts using lysine- and cysteine-labeling two-dimensional difference gel electrophoresis. J Proteomics 75:1991–2014

Zhang L et al (2000) Role of BAX in the apoptotic response to anticancer agents. Science 290:989–992

Zhang A et al (2012) Modern analytical techniques in metabolomics analysis. Analyst 137:293–300