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Review

Proteomics in uveal melanoma

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ABSTRACT

Uveal melanoma is the most common primary intraocular malignancy in adults, with an incidence of 5–7 per million per year. It is associated with the development of metastasis in about 50% of cases, and 40% of patients with uveal melanoma die of metastatic disease despite successful treatment of the primary tumour. The survival rates at 5, 10 and 15 years are 65%, 50% and 45% respectively. Unlike progress made in many other areas of cancer, uveal melanoma is still poorly understood and survival rates have remained similar over the past 25 years. Recently, advances made in molecular genetics have improved our understanding of this disease and stratification of patients into low risk and high risk for developing metastasis. However, only a limited number of studies have been performed using proteomic methods. This review will give an overview of various proteomic technologies currently employed in life sciences research, and discuss proteomic studies of uveal melanoma.

1. Introduction

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults. The overall incidence is approximately 5–7 cases per million per year, and climbs to more than 20 cases per million per year by the age of 70 (Egan et al., 1988; Singh and Topham, 2003). It is more common in the Caucasian population, especially those with blue/grey iris. The survival rates at 5, 10 and 15 years are 65%, 50% and 45% respectively (Jensen, 1982; Raivio, 1977; Singh and Topham, 2003). Ninety-two percent of cases of UM arise in the choroid while the remainder arise in the iris (3%) and ciliary body (5%). Choroidal melanomas have the worst prognosis, while iris melanomas have the most favourable. The latter may be due to earlier presentation, as iris melanomas may be detected by the patient externally. Unfortunately, choroidal melanomas are usually detected late, when the patient is symptomatic with decreased visual acuity. This may be due to the involvement of the macula, or when complicated by secondary retinal detachment. There are several treatment options available for uveal melanoma. There are several treatment options available for uveal melanoma. These include eye-preserving plaque radiation brachytherapy, proton beam therapy, local resection, and endoresection. However, many patients require or opt for enucleation due to a large tumour size at presentation (Damato, 2012).

Uveal melanoma is associated with the development of metastasis in about 50% of cases, and 40% of patients with UM die of metastatic disease despite successful treatment of the primary tumour (Bedikian, 2006; Ehlers and Harbour, 2006). Metastatic spread occurs haematogenously, predominantly to the liver in up to 90% of patients with metastatic disease (Singh and Borden, 2005). Other potential sites include lung, bone and skin, but these are rare in the absence of liver metastasis (Lorigan et al., 1991). The occurrence of metastasis is primarily detected after disease-free intervals following local treatment, sometimes after more than a decade. This suggests the presence of occult micrometastatic disease at the

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Abbreviations

GNAQ	guanine nucleotide binding protein (G protein), q polypeptide[1]
GNA11	guanine nucleotide binding protein (G protein), alpha 11 (Gq class
BAP1	BRCA1 associated protein-1 (ubiquitin carboxy-terminal hydrolase)
SF3B1	splicing factor 3b, subunit 1
2D-PAGE	two-dimensional polyacrylamide gel electrophoresis
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
2D-DIGE	two-dimensional difference gel electrophoresis
MALDI	matrix-assisted laser desorption/ionization
ESI	electrospray ionization'
Q-TOF	quadrupole-time of flight
MS	Mass spectrometry
CID	collision induced dissociation
SID	surface induced dissociation
ECD	electron capture dissociation
ETD	electron transfer dissociation
SILAC	Stable Isotope Labelling by Amino acids in Cell culture
ICAT	isotope-coded affinity tags
iTRAQ	Isobaric Tag for Relative and Absolute Quantification
LC	Liquid chromatography
TMT	Tandem mass tags
AQUA	absolute quantification of proteins
SRM	selected reaction monitoring
MRM	multiple reaction monitoring
HSP	Heat shock protein
BRCA-1	breast cancer type 1 susceptibility protein
HMG-1	high-mobility group protein 1
MUC18	Cell surface glycoprotein MUC18
DJ-1	Parkinson disease (autosomal recessive, early onset) 7/ PARK7
PAK1	p21-activated kinase 1
FABP3	fatty acid-binding protein, heart-type
TPI1	triosephosphate isomerase
PFKM	phosphofructokinase, muscle
LDHB	Lactate dehydrogenase B
PHB	Prohibitin

time of the diagnosis and treatment of the primary eye tumour (Eskelin et al., 2000). When liver metastasis is diagnosed, treatment options are limited and survival is short, averaging 5–8 months (Triozzi et al., 2008). From the onset of symptoms from hepatic metastases, the median survival is between 3 and 6 months (Eskelin et al., 2003; Kim et al., 2010). Despite progress in early diagnosis and conservation of vision, mortality rates have remained similar over the last 25 years (Jemal et al., 2006; Singh et al., 2005).

There are several prognostic factors which include clinical, histopathologic and cytogenetic factors, the latter being the most accurate and reliable. Until recently, cell type was the most used prognostic indicator as epithelioid tumours are more aggressive than spindle cell type. Recent advances in molecular genetics have increased our knowledge on the cytogenetic properties of uveal melanoma. Cytogenetic and molecular genetic studies have revealed that deletion or loss of heterozygosity of chromosome 3 and gain of chromosome 8 correlates with an increased risk of metastasis (Damato et al., 2010, 2007; Thomas et al., 2012). A number of gene-expression profiling studies have revealed that primary UM clusters in two different classes; class 1 tumours that are associated with a good prognosis and class 2 tumours with a high metastatic risk (Onken et al., 2004; Tschentscher et al., 2003). The most common known oncogenic mutations occur in GNAQ or GNA11 which are found in about 85% of all primary UM, irrespective of tumour class or stage (Onken et al., 2008; Van Raamsdonk et al., 2010). These mutations may represent an early event that leads to the development of UM. Further downstream, mutations in BAP1 gene located in chromosome 3, were found to occur almost exclusively in metastasizing class 2 tumours (Harbour et al., 2010). It is by Harbour et al. that either BAP1 inactivating mutations or chromosome 3 loss can occur first, but both events are thought to be necessary for the tumour to metastasise (Harbour, 2012). More recently, mutations in splicing factor SF3B1 were found to be associated with a better prognosis (Harbour et al., 2013). Individuals with SF3B1-mutant tumours tended to have a lower metastasis rate than those with tumours with wild-type SF3B1. SF3B1 and BAP1 mutations were almost mutually exclusive, suggesting that they may represent alternative pathways in tumour progression (Harbour et al., 2013). In a distinct, atypical subset of uveal melanoma, Lake et al. (2013) recently identified amplification of CNKSR3 in tumours with monosomy 3 that did not metastasise. Little is known about the function of this gene, but the authors hypothesise that this gene may play a role in limiting metastatic development.

Gene expression profiling/transcriptomic studies identify an intermediate carrier (mRNA) of the genetic information between the genome and proteome. However, genetic and transcriptomic studies alone are not sufficient to fully understand the molecular basis for the association between these cytogenetic alterations and aggressive phenotype, with several investigators reporting a poor correlation between mRNA and protein abundance (Gygi et al., 1999b; Mádi et al., 2003). This is due to the fact that a single gene can encode for more than one mRNA species through differential splicing, and proteins can undergo as many as 200 post-translational modifications (Srinivas et al., 2001). The regulatory role of micro-RNAs in gene expression at the post-transcriptional level adds to the limitations of genetic studies (Fabian et al., 2010). While genomics is significantly improving our understanding of the molecular basis of this disease, identifying targets suitable for treatment is difficult. Pharmacologic targeting of genetic mutations is complex and challenging. Direct inhibition of mutant GNAQ or GNA11may prove difficult because these mutations abrogate the intrinsic GTPase activity that would normally allow these proteins to return to their GDP-bound, inactive state (Harbour, 2012). Loss of BAP1 also poses a difficult therapeutic challenge, as it is seems to represent a classic loss of a tumour suppressor, and direct therapies would require the reinitiation of function (Woodman, 2012). Proteomics delineates the functional units of a cell, proteins and their intricate interaction network and signalling pathways for the underlying disease (Boja and Rodriguez, 2011). Proteomic studies have been successfully used to identify several protein alterations in tumour cells, leading to biomarker discoveries (Celis et al., 1998; Desmetz et al., 2011; Emmert-Buck et al., 2000; Guo et al., 2011). Remarkable progress has been made in several areas of cancer research using proteomics technology, including breast (Baskın and Yiğitbaşı, 2010), lung (Hassanein et al., 2011), oral (Mehrotra and Gupta, 2011), and colorectal cancers (Bosch et al., 2011).