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REVIEW ARTICLE

Genomic and proteomic findings in early melanoma and opportunities for early diagnosis

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Abstract

Overdiagnosis of early melanoma is a significant problem. Due to subtle unique and overlapping clinical and histological criteria between pigmented lesions and the risk of mortality from melanoma, some benign pigmented lesions are diagnosed as melanoma. Although histopathology is the gold standard to diagnose melanoma, there is a demand to find alternatives that are more accurate and cost-effective. In the current "omics" era, there is gaining interest in biomarkers to help diagnose melanoma early and to further understand the mechanisms driving tumor progression. Genomic investigations have attempted to differentiate malignant melanoma from benign pigmented lesions. However, genetic biomarkers of early melanoma diagnosis have not yet proven their value in the clinical setting. Protein biomarkers may be more promising since they directly influence tissue phenotype, a result of by-products of genomic mutations, posttranslational modifications and environmental factors. Uncovering relevant protein biomarkers could increase confidence in their use as diagnostic signatures. Currently, proteomic investigations of melanoma progression from pigmented lesions are limited. Studies have previously characterised the melanoma proteome from cultured cell lines and clinical samples such as serum and tissue. This has been useful in understanding how melanoma progresses into metastasis and development of resistance to adjuvant therapies. Currently, most studies focus on metastatic melanoma to find potential drug therapy targets, prognostic factors and markers of resistance. This paper reviews recent advancements in the genomics and proteomic fields and reports potential avenues, which could help identify and differentiate melanoma from benign pigmented lesions and prevent the progression of melanoma.

KEYWORDS
biomedical, diagnostic, genomics, melanoma, proteomics

Abbreviations: CAD, computer-assisted devices; CGH, comparative genomic hybridisation; CNA, copy number aberration; ctDNA, circulating tumor DNA; DDA, data-dependent acquisition; DIA, data-independent acquisition; FFPE, formalin-fixed paraffin-embedded; FISH, fluorescent in situ hybridisation; HGMS, histopathology-guided MS–MALDI-TOF MS; IDA, information-dependent acquisition; IHC, immunohistochemistry; LC–MS/MS, liquid chromatography–tandem mass spectrometry; MRM/SRM, targeted multiple or selective reaction monitoring; MSI, mass spectrometry imaging; NGS, next-generation sequencing; NVP, negative predicted value; PLA, pigmented lesion assay; WB, western blot.

1 | INTRODUCTION

Melanoma is a lethal form of skin cancer worldwide but especially in Australia. As of 2021, the Australian Institute of Health and Welfare estimated over 16 000 new cases of melanoma in the population, with an estimate of 55.3 per 100 000 persons at an age-standardised incidence rate.[1] Further, an estimated mortality rate for melanoma is reported at 1315 deaths or 4.0 per 100 000 at the age-standardised rate.[1] The current paradigm for the management of possible melanomas is the excision and histopathology analysis of the detected lesion. However, if a melanoma is missed, survivability declines with increasing tumor size. In a 20-year follow-up study, Baad and colleagues (2020) reported 0.4% of patients with tumors <1 mm thick compared to 45% of patients with tumors >6 mm thick succumbed to the disease.[2] Additionally, therapies for metastatic melanoma had incredible success recently, but still 50% of patients were lost to the disease in 3 years, despite costly drug treatment.[3] In this regard, early diagnosis and excision remains the primary standard of care. However, this emphasis has led to an increase in both unnecessary excisions and melanoma in situ diagnoses, without a reduction in invasive malignant melanoma cases.[4] Improvements in early diagnosis are needed to make the detection of early melanoma cases more efficient.

2 | CLINICAL AND HISTOPATHOLOGY FEATURES CANNOT ACCURATELY DISTINGUISH BETWEEN PIGMENTED LESIONS

Melanoma diagnosis first requires clinical inspection (ABCDE criteria, the Menzies method and Ugly Duckling) of the lesion.[5,6] The ability to distinguish lesions can take many years of training and experience and even then, with overlapping features and lack of clear discriminative features, results in approximately one melanoma diagnosed for every 2–200 surgeries.[7] To confirm the clinical diagnosis, pathologists study the histopathological sample and evaluate characteristics such as number, distribution and shape of melanocytes/melanoma cells, presence of invasion in the dermis, and tissue reaction. If the lesion is classified as melanoma, criteria such as Breslow thickness, ulceration, regression, irritation, mitotic rate, presence of immunocytes and more features are used to determine the severity of the diagnosis.[8] Here, information missed, omitted or incorrectly observed can detrimentally affect the patient due to overcalling or undercalling the diagnosis of melanoma.[9] Precise preparation of biopsy specimens as well as sufficient clinical information is critical for histopathological assessment.[9] However, despite attempts to improve diagnosis, accurate assessment of pigmented lesions and melanoma remains difficult.[10]

Strategies to remove every lesion with malignant potential are extreme and ineffective, since only a minority (30%) of melanomas develop from a pre-existing naevus, the majority developing de novo.[11] Surgical interventions also come with an inherent risk of infections, scarring, and depending on the size and anatomical location may require extensive cosmetic reconstruction procedures.[12,13] Liu and colleagues (2018) recently reported an increased risk of surgical site infections of the ear and large defects or wounds in an analysis of 1977 procedures in 1407 patients.[13] Importantly, mental health and quality of life decline, a study conducted in 1939 patients where 36% reported body image as a contributing factor to depression (average score was 30% higher than those who did not report appearance as a problem) and anxiety (average score was 25% higher than those who did not report appearance as a problem) in surgical treatment of melanoma.[12]

Although benign lesions are most accurately diagnosed (92% concordance between pathologists),[10] accuracy decreases with minimally invasive melanoma diagnosis (72% concordance) and it is worse with intermediate lesions such as melanoma in situ (40%) and dysplastic naevi (25%).[10] Specifically, intermediate lesions are the most common cause for controversy and are where most disparity occurs due to shared histological characteristics of both benign naevi and malignant melanoma; therefore, they can be missed and potentially progress to melanoma.[11] This histological difficulty may contribute to the current rise in melanoma in situ diagnosis.[4]

The exact mechanism that drives the progression of a benign lesion towards malignancy is not completely understood. Thus, to know exactly which lesion will become malignant is still a mystery.[14] A multilevel comprehensive analysis of melanoma and its associated pigmented lesions would help to understand melanoma and its mechanisms of progression and would contribute to improved diagnosis of melanoma (Figure 1).

3 | GENOMICS AS A FOUNDATION FOR IN-DEPTH INVESTIGATION INTO MELANOMA AND ITS PRECURSORS

Many studies investigating the genome of melanoma and its associated lesions have helped to uncover genomic aberrations, mutational processes and heterogeneity in the development of this disease.[14] Moreover, these studies have provided insight into the progression of melanoma, its response to therapy and treatment, as well as its prognosis.[18]

Genomic techniques such as comparative genomic hybridisation (CGH), fluorescent in situ hybridisation (FISH) and next-generation sequencing (NGS) have uncovered mutations from benign naevi to metastatic melanoma. Table 1 summarises the most recent genomic research in melanoma. Primarily, genomic studies have found that a percentage of melanomas (~50%) carry $BRAF^{V600E}$, which affect the mitogen-activated protein kinase (MAPK) signalling pathway resulting in uncontrolled proliferation of cells.[18,19] As a result, BRAF inhibitors were developed, for example, vemurafenib, to treat metastatic melanoma.[20] Though treatment has shown a successful response in more than 50% of patients, some patients eventually develop resistance.[20]

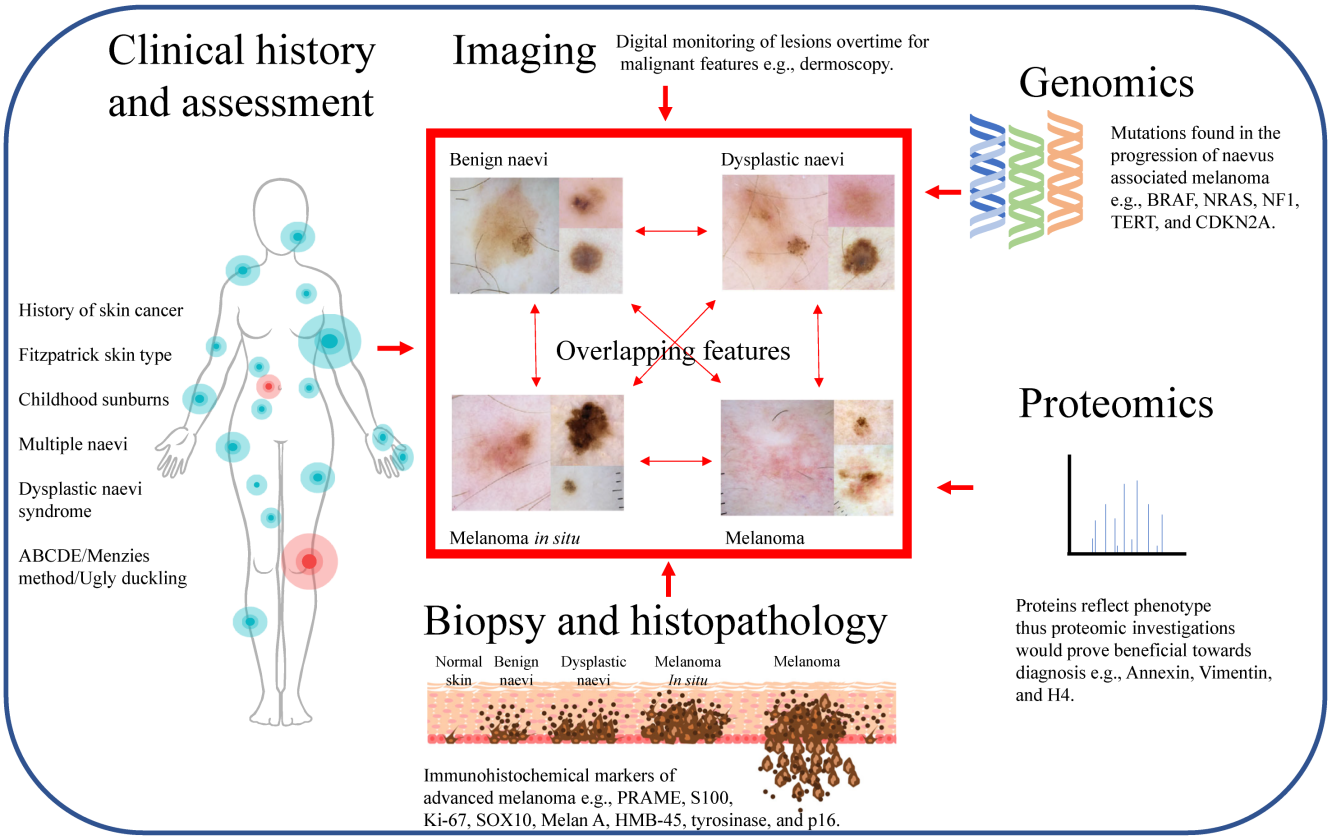

FIGURE 1 Overview of multilevel approach towards improving melanoma diagnosis. Images obtained from Sydney Melanoma Diagnostic Centre, Royal Prince Alfred Hospital, NSW, Australia, of histopathology confirmed lesions that were suspect of melanoma due to malignant features revealed by dermoscopy or total body photography. Current examples of the tools utilised to diagnose melanoma include clinical history and assessment (personal and family history of melanoma, Fitzpatrick skin type, childhood sunburns, multiple naevi, dysplastic naevi syndrome, identification of suspicious lesions, for example, ABCDE/Menzies method/Ugly duckling); imaging, for example, dermoscopy; immunohistochemical markers for melanoma diagnosis; genomics, for example, BRAF, NRAS, NF1, TERT, CDKN2A[15]; proteomics, for example, Annexin, Vimentin, H4 are some of the proteins so far commonly found.[16,17]

Resistance to therapeutic treatments drives the need for further study into the mechanisms of resistance and alternative therapies. As such, a study using whole-genome sequencing of 183 melanoma tumor and cell lines comparing cutaneous, acral and mucosal subtypes demonstrated significant differences between cutaneous melanoma and the other subtypes (acral and mucosal melanoma).[21] Acral and mucosal melanomas were lower in mutation burden but contained large-scale structural variants compared with cutaneous subtypes. Also, UVR was not the overall driving mutation mechanism in mucosal and acral subtypes whereas most cutaneous melanoma had UVR signature representation. The study revealed the use of markers of mutagenesis and structural changes towards uncovering the heterogeneous mechanisms of the mutations.[21] The existence of varying subtypes is a factor for the difficulties faced with diagnosis, differing treatment responses and prognosis. Finally, a multidiscipline study involving genomic and transcriptomic techniques demonstrated a consecutive order of mutations (signalling pathways involved in MAPK, telomerase, cell cycle, chromatin, p53 and PI3K) that occur in the progression to malignant melanoma suggesting an evolution from premalignant lesions as well as possible biomarkers.[14]

Nevertheless, current genomic investigations have not validated a reliable marker let alone signature for clinical use to classify early melanomas from benign lesions.[18] Deacon, Smith and Judson-Torres (2021) recently reviewed prospective diagnostic markers and signatures (eg PRAME, LINC00518, PMEL, RASSF1A, cfDNA, myPATH gene expression panel, copy number variation assay, among others) where they highlight the infancy of the currently assessed markers and the need for further validation before adoption in the clinical setting.[18] On the contrary, in advanced melanoma, there are signatures (eg CAM-121 of 121 differentially expressed genes) that predict the risk of metastasis and progression with very high confidence,[37] and NGS panels for circulating tumor DNA (ctDNA) that may stratify patients who will and will not respond to therapy (eg Clinical trial phase II BREAK-2 for BRAF inhibitor dabrafenib targets BRAF-V600E where high levels correlated with low response rate).[38] Liquid biopsies, which detect biomarkers such as ctDNA in bodily fluids such as blood, might also have the potential as an early diagnostic tool with advantages in cases where surgical biopsy is not desired (uveal melanoma of the eye).[38,39] However, liquid biopsy as an early diagnostic tool is in its infancy and requires validation and reproducibility studies

TABLE 1 Summary of recent genomic studies in benign pigmented lesions and melanoma between 2015 and 2021

Year (reference)	Sample type	Lesion type	Genomic method	Study findings/implications
2021[22]	Genome-wide association study data; blood, serum, peripheral blood mononuclear cells, peripheral blood leukocytes, buccal, saliva, FFPE and fresh frozen.	Cutaneous melanoma; cases ($n = 36,760$), control ($n = 375,188$), effective cases ($n = 49\,274$) and effective controls ($n = 49\,274$).	Meta-analysis of genome-wide association studies.	Reported 68 independent cutaneous melanoma-associated variants across 54 known and previously unknown loci confirming key functional pathways with melanoma risk.
2020[23]	Fresh frozen	Benign naevi ($n = 8$), non-metastatic primary melanoma ($n = 8$) and metastatic primary melanoma ($n = 8$).	DNA methylation profiling array	Reported lower levels of 5-hydroxymethylcytosine (hmC) in melanoma (0.54% of interrogated CpGs) compared with naevi (2.0%) across 743016 CpG sites.
2020[24]	FFPE	Benign naevi ($n = 66$) and melanoma ($n = 98$).	RNA sequencing	Identified eight miRNA relative expression ratios of six miRNAs (MIR31-5P; MIR21-5P; MIR211-5P; MIR125A-5P; MIR125B-5P; MIR100-5P), which could differentiate naevi from melanoma.
2020[25]	FFPE tissues and peripheral blood.	Normal ($n = 27$), primary ($n = 22$), malignant melanoma ($n = 17$).	Whole-genome sequencing	Genetic mutation profiles were consistent between primary and metastatic melanoma in Chinese patients. Identified germline mutations, copy number variations and somatic mutations different in Chinese patients from previous studies which focussed on western populations.
2019[26]	Fresh frozen, blood.	Benign naevi ($n = 18$).	Whole-genome sequencing	Confirmed BRAFV600E, NRAS Q61R and NRASQ61K mutations with an overall low mutational load in naevi. Mutational signatures can subdivide naevi into a UVR high group predominant in signatures 7a and 7b, while a UVR low category was predominantly associated with signatures 1 and 5. Identified some TERT promoter mutations in acquired naevi subclones.
2018[27]	Microarray data, DNA methylation data; cell lines.	Human melanocyte ($n = 9$) and primary melanoma ($n = 12$).	mRNA expression and DNA methylation profiles of microarray data.	Identified 1215 differentially expressed genes and 14094 differentially methylated positions. Identified methylated genes aristaless-related homeobox (ARX), damage-specific DNA binding protein 2 (DDB2) and myelin basic protein (MBP) potentially involved in the development of melanoma.
2018[28]	Genome-wide single-nucleotide polymorphism genotype data; blood, urine, hair, saliva, placenta and plasma.	Naevi ($n = 52\,506$), melanoma ($n = 12\,874$), and control ($n = 23\,203$).	Genome-wide single-nucleotide polymorphism genotype meta-analysis	Reported 30 regions containing SNPs associated with naevus count and/or melanoma risk; of which eight were novel (DOCK8, KITLG, GPRC5A, HDAC4, FMN1, FAM208B, SYNE2 and NFIC).

(Continues)

TABLE 1 (Continued)

Year (reference)	Sample type	Lesion type	Genomic method	Study findings/implications
2018[29]	Fresh frozen	Benign naevi ($n = 23$) and primary melanoma ($n = 80$).	RNA sequencing	Identification of two naevus (N) and melanoma (M) subsets; Type 1 (N1/M1) and Type 2 (N2/M2). Type 1 predominant in gene signatures involved in pigmentation and MITF, and NRAS mutations; Type 2 predominant in gene signatures involved in inflammation and AXL, and wild-type and mutated BRAF with low NRAS mutation prevalence. Further, M1 contained BRAF/MEK inhibitor resistance signatures, while M2 contained transcriptional signatures involved in anti-PD-1 antibody treatment resistance.
2018[14]	FFPE	Total patients $n = 47$; normal, benign naevi, intermediate lesions, melanoma in situ, malignant melanoma.	DNA and RNA sequencing.	Report a sequential order of altered signalling pathways from early benign naevi to melanoma (including any mutated gene involved in MAPK, any telomerase, any cell cycle, any chromatin, multiple strong MAPK, multiple cell cycle, any p53 and finally any PI3K).
2018[30]	Fresh tissue, fresh frozen, FFPE and saliva.	Benign ($n = 18$) and dysplastic ($n = 12$) naevi ($n = 30$) with matched normal	Whole-exome sequencing	Reported signatures 7 (UVR), 1 (age-related), then 20 and 6 (DNA mismatch repair) as prevalent mutations in naevi. Also, minor copy number aberrations (CNA) were seen in globular naevi, compared with reticular/non-specific naevi. Further, CNA events were balanced in reticular/non-specific naevi suggesting random mutagenic processes rather than targeted.
2017[31]	Blood	Non-cancer controls ($n = 11\,192$), cutaneous ($n = 652$) and ocular melanoma ($n = 146$).	Whole-exome sequencing	First exome-based study focussing on rare germline variants associated with melanoma in cutaneous and ocular melanoma patients, for example, rare coding mutations were identified such as protein truncating variants in CDKN2A and BAP1; novel non-sense (p.Arg363X) and splice donor mutations in POT1, and MC1R which revealed 184 alternative alleles. EBF3 was proposed as a predisposition gene.
2017[21]	Derived cells, fresh frozen and blood.	Cells derived from metastases ($n = 15$), primary ($n = 75$) and metastatic melanoma ($n = 93$).	Whole-genome sequencing	Identified 12 mutation signatures; Signature 7a, 7b and 7c were associated with UVR; the remaining not associated with UVR (signature 5 and 17 with unknown aetiology). Whole-genome mutational differences were seen comparing cutaneous, acral and mucosal melanoma, for example, cutaneous melanoma mutation signatures characterised by UVR while acral and mucosal were not.
2017[32]	Fresh tissue, FFPE and matching peripheral blood lymphocytes.	Discovery cohort: 22 patients; congenital melanocytic naevi ($n = 4$), common acquired naevi ($n = 8$), dysplastic naevi ($n = 23$), Extension cohort: total patients ($n = 22$); common acquired naevi ($n = 30$), dysplastic naevi ($n = 16$), and primary melanoma ($n = 42$).	Whole-exome sequencing	Reported dysplastic naevi with lower mutational load (non-synonymous mutations) than melanoma (21 and >100). $BRAF^{V600E}$ and $NRAS^{Q61K/R}$ mutations were identified in naevi however CDKN2A, TP53, NF1, RAC1 and PTEN were not found in naevi of the study The most frequent substitution variants were C>T, CC>TC, TC>TT, and CC>CT. TC>TT was differentiating in primary melanomas compared with naevi while CC>CT was not significantly differentiating.

TABLE 1 (Continued)

Year (reference)	Sample type	Lesion type	Genomic method	Study findings/implications
2017[33]	Fresh frozen, gene expression datasets (GEO database) and FFPE.	Discovery cohort: Benign naevi ($n = 14$), primary melanoma ($n = 33$) and metastatic ($n = 28$). Validation cohort I: primary melanoma ($n = 19$) and metastatic ($n = 23$). Validation cohort II: primary melanoma ($n = 85$). Validation cohort III: primary melanoma ($n = 179$).	Whole-genome DNA methylation	Identified 1533 genes significantly hypermethylated in melanoma vs benign samples targeting CpG islands, and 1722 genes with no association with CpG islands but occurring in isolated CpGs in the genome. The genes AKT3, EPHX3, GJB2, HOXA9, MEOX2, PON3, RBP1, SERPINE2, TBC1D16, TFAP2B and TWIST1 were validated as melanoma progression. MEOX2, OLIG3 and PON3 to predict prognosis.
2017[34]	Cell line	HMEL-BRAF^{V600E}, PMEL-BRAF^{V600E}, Hs839.T, Skmel-28, Skmel-5, WM115 and WM793B.	Epigenome profiling	Epigenomic profiling of non-tumorigenic and tumorigenic phenotypes, for example, revealed chromatin states with acetylation and H3K4me2/3 methylation are lost in tumorigenic phenotypes. Chromatin state changes in transition to TM involved in tumorigenic processes such as proliferation, apoptosis and adhesion.
2016[35]	Transcription data; fresh frozen and cell lines.	Normal ($n = 103$), naevi ($n = 21$) and primary melanoma ($n = 132$), and metastatic melanoma ($n = 222$).	Transcriptional profiling	Identified 25 metabolic and 19 signalling pathways to discriminate between normal skin, naevi and melanoma, of which 6 metabolic pathways were novel associations with melanoma progression; Allopregnanolone biosynthesis, L-carnitine biosynthesis, Zymosterol biosynthesis, D-myo-inositol hexakisphosphate biosynthesis, Fructose 2, 6-bisphosphate synthesis and dephosphorylation, Resolvin D biosynthesis.
2015[19]	FFPE	Total samples ($n = 37$); benign naevi, intermediate lesions and malignant melanoma. A total of 37, 150 areas were selected.	DNA sequencing	Successive genetic alterations such as point-mutation burden in the development of melanoma from benign naevi. Benign naevi harboured BRAFV600E mutations; intermediate harboured NRAS, intermediate and melanoma in situ with TERT promoter mutations; CDKN2A for invasive and PTEN and TP53 were found in advanced primary melanomas. CNA were also only prevalent in invasive melanomas. UVR role is evident from naevus development to melanoma.
2015[36]	Fresh frozen and blood.	Primary ($n = 67$) and metastatic melanoma ($n = 266$).	Multiple platforms used: whole-exome sequencing, DNA copy number and SNP array whole-genome sequencing, RNA sequencing, DNA methylation, reverse phase protein array and microRNA sequencing.	Identified 228, 987 mutations. Classification of four genomic subtypes (mutant BRAF, mutant RAS, mutant NF1 and Triple-wild-type) based on most occurring mutated genes. Transcriptomic classification of three clusters (immune, keratin and MITF-low) which has the potential to be markers for prognostic and treatment response prediction.

to demonstrate robustness of selected biomarkers. Genomic research has contributed to a significant breadth of information; however, complementing these studies in the other "OMIC" fields would further enrich our understanding of melanoma.[40]

4 | PROTEOMICS AND ITS POTENTIAL TO UNCOVER DIFFERENTIATING BIOMARKERS AND GIVE INSIGHT INTO PHENOTYPE

Proteomics is the large-scale study of proteins. The genome and all its alterations and modifications are reflected in the proteome and thus executors of a cell's phenotype.[41] Therefore, it can be argued that proteomics is more relevant in studying tumor phenotype.[41] Presently, there are limited proteomic studies focussing on pigmented lesions and studies using proteomic techniques for melanocytic cancer research are in its infancy.

Traditionally, small-scale techniques such as immunohistochemistry (IHC) and western blot (WB) were routinely used to identify and quantify proteins of interest in a tissue sample. However, they are restricted to prior knowledge of proteins of interest as well as available and reliable antibodies to detect them.[42] Some IHC protein biomarkers for melanoma diagnosis include S100 family (markers of advanced melanoma), Ki-67 which is a marker for proliferation and linked to prognosis, as well as preferential expressed antigen in melanoma (PRAME), HMB45, SOX10 among others reviewed by Hessler and colleagues 2020.[15] Techniques like IHC are limited to a few individual proteins, but these are easily accessible and relatively inexpensive. However, there are currently no widely used IHC biomarkers for the early detection of melanoma in the clinical setting. Further, heterogeneity of melanomas and benign pigmented lesions suggests that the absence or presence of one biomarker may not be sufficient to identify malignancy.[43] For example, HMB-45 has a broad sensitivity range between 66% and 97% and this sensitivity declines in metastatic lesions compared with primary. Another example is melan-A, which cannot discriminate between melanoma in situ and keratoses. Additionally, the high rate of mutations in melanoma suggests that it could take a multitude of proteins to drive progression into malignancy.[14]

As such, biomarker signatures would include more than one protein to represent the heterogeneity. High-throughput techniques such as protein microarrays provide a deeper investigation with the ability to analyse hundreds of proteins at once as well as provide information about differential expression within a sample.[44] Zaenker and colleagues (2018) developed a 10 autoantibody signature that could potentially detect primary melanomas early.[45] Alternatively, mass spectrometry is a method, which investigates proteins in all its complexity and provides in-depth protein coverage.

Revolutionary technologies such as mass spectrometry have improved the capacity to investigate proteins on a large scale.[46] Mass spectrometry is an analytical approach that measures proteins proteolytically digested and extracted.[46] To explain simply, a sample is run through several components of the machine each with an overall purpose in detecting protein relative abundance: an ion source, a mass analyser and a detector. The acquired data are bioinformatically analysed to identify and quantify proteins.[46] There are several workflows that could be utilised. The three main methods include data-dependent acquisition (DDA/IDA), targeted multiple or selective reaction monitoring (MRM/SRM) and data-independent acquisition (DIA).

At present, there are a limited number of studies that use proteomic techniques in the study of early melanoma (Table 2). In 2014, Qendro and team (2014) conducted the first large-scale study in both early and malignant stages of melanoma.[16] The study compared benign with metastatic tissue cell lines and identified over 4500 proteins. A total of six (vimentin, nestin, fibronectin, annexin A1, dipeptidyl peptidase IV and histone H2A1B) potential candidate biomarkers were selected for further investigation for prognosis and treatment therapies. The next year, Guan (2015) used a combined two-dimensional gel electrophoresis gel (2-DE) and mass spectrometry to identify potential differences between cutaneous melanoma and metastatic melanoma as well as biomarkers to predict malignancy.[47] As a result, they reported 2 markers, MDA-9 co-expressed with GRP78, as correlating with malignancy. Studies such as these give the groundwork for further developing our understanding of melanoma progression, the pathways and biological processes potentially affected at each stage as well as subtype, and biomarkers for clinical use.

The studies summarised in Table 2 from 2003 until 2021 focus on finding differential proteins or markers that suggest melanoma progression and their pathways. A comprehensive review by Sengupta and colleagues (2016)[48] reported on 25 proteomic studies contributing to melanoma research from 2011 to 2016. Most studies have focussed on metastatic melanoma (Table 2), for example, Shapanis (2020)[49]; while only four studies include benign naevi,[50,51] dysplastic naevi[52] and melanoma in situ.[53] Moreover, the use of formalin-fixed paraffin-embedded (FFPE) samples as a reliable source of material for proteomic investigations had only been recently demonstrated.[54–58] For instance, Shields and team (2016) gave a perspective on the advantages and limitations proteomics combined with sample types such as FFPE have in melanoma research.[59] Previously, hesitancy in FFPE usage was attributed to issues with fixation and cross-linking of proteins limiting comprehensive analysis.[58] However, improvements in sample preparation combined with the availability of FFPE tissue samples create a valuable resource avenue for research. Finally, studies have mostly made use of DDA and targeted proteomics, with the use of DIA in only one study so far.[40] Gao and colleagues (2021) identified over 8000 proteins in primary and metastatic melanoma cell lines and revealed the sensitivity and reproducibility of DIA liquid chromatography–tandem mass spectrometry (LC–MS/MS) applications in the investigation of melanoma proteomes.[40] Interestingly, DIA methodology provides the capacity to acquire data on a large number of proteins similar to DDA while maintaining the sensitivity of targeted mass spectrometry.[46] Importantly, the bias from detecting largely abundant proteins is reduced.[46] These features are further enhanced by the use of specialised in-house spectral ion libraries.[60]

TABLE 2 Summary of recent proteomic studies in benign pigmented lesions and melanoma between 2003 and 2021

Year (reference)	Sample type	Lesion type	Proteomic method	Study findings/implications
2021[61]	FFPE, fresh frozen, cell lines and blood (plasma, serum, lymphocytes, erythrocytes).	Malignant melanoma cell lines (SK-MEL-2, SKMEL28 and VMM1), malignant melanoma samples ($n = 505$).	DDA and DIA LC-MS/MS.	Identification 13 176 protein-coding genes correlated with 366 172 peptides, 52 000 phosphorylation sites and 4400 acetylation sites. The study also reported 12 751 gene products overlap with TCGA transcriptomic data of melanoma.
2021[17]	FFPE	Benign naevi ($n = 102$) and malignant melanoma ($n = 101$).	Mass Spectrometry Imaging (MSI)	Classification model of 18 and 187 peptides differentiated benign naevi from malignant melanoma with an overall accuracy of 93% in an independent dataset.
2021[40]	Cell lines	Primary melanoma (TCP-1013, SK-MEL-1, A375, G-361), metastatic melanoma (TCP-1014, SK-MEL-3, SH-4, RPMI-7951).	DIA LC-MS/MS	Assessment of DIA as a reliable tool to investigate proteomic and phosphoproteomic signatures in cell lines. Study found up to 8100 proteins.
2020[49]	FFPE	Primary melanoma non-metastasised ($n = 24$), metastasised ($n = 24$).	LC-MS^E	16 differentially abundant proteins found between metastasised and non-metastasised primary melanoma samples out of 2750 proteins identified.
2020[62]	FFPE	Benign naevi ($n = 399$) and malignant melanoma ($n = 357$).	Histopathology-guided MS–MALDI-TOF MS (HGMS)	Classification model using HGMS proteomic signature was able to distinguish between benign naevi and malignant melanoma.
2019[41]	Cell lines and fresh frozen.	Malignant melanoma SK-MEL-2, SKMEL28 and VMM1, primary ($n = 11$) and lymph node metastatic melanoma (9).	DDA LC-MS/MS	Quantitation of over 6000 proteins. Study found protein variability between primary tumor samples while lymph node metastatic tumors and cell lines were well distinguished and clustered via PCA.
2019[63]	Cell line	Primary uveal melanoma (Mel202).	DDA LC-MS/MS	Study identified 2527 unique proteins. Suggests ectosomes as a potential source for uveal metastatic melanoma markers.
2019[64]	FFPE	Primary melanoma ($n = 14$).	DDA LC-MS/MS	Identified 4006 protein groups and correlates mutational status with proteomic differences in FFPE samples by identifying 17 proteins differentially expressed between BRAF-mutant and NRAS-mutant and double-negative.
2018[45]	Serum	Control ($n = 111$) and malignant melanoma ($n = 124$).	Protein Microarray	A biomarker signature of 10 autoantibodies that could be used to identify early melanoma in serum.
2018[65]	Fresh tissue and FFPE.	Total patients ($n = 15$); cerebral metastatic melanoma.	DDA LC-MS/MS and targeted MRM.	Reported mechanisms involved in MAPKi resistance as well as potential predictive markers of cerebral metastasis. Identified 5977 proteins with epithelial-mesenchymal transition (EMT) as a classifier between good and poor prognosis. In poor responders, V-type proteins, cell adhesion, transporter and exchanger proteins were upregulated, while good responders showed immune activation.
2017[66]	Case report FFPE	Total patient ($n = 1$); benign congenital naevus and metastatic melanoma.	Mass Spectrometry Imaging (MSI)	Using an algorithm previously distinguishing spitz naevus from spitzoid melanoma, demonstrated potential usage of MSI to differentiate between benign congenital naevus and malignant melanoma in a single case study.
2017[67]	Serum	Control ($n = 16$) and malignant melanoma ($n = 165$).	Immunocapture mass spectrometry (IC-MS)	Identification of potential serum proteins RGN, STX7, MTHFD1L and S100A6 suggestive of melanoma progression.

(Continues)

TABLE 2 (Continued)

Year (reference)	Sample type	Lesion type	Proteomic method	Study findings/implications
2016[53]	Serum	Control ($n = 100$), melanoma in situ ($n = 45$) and malignant melanoma ($n = 303$).	DDA LC-MS/MS	Serum vitronectin and dermcidin potentially predict the progression of metastatic melanoma and prognosis.
2016[68]	FFPE	Melanoma in situ ($n = 5$) and malignant melanoma ($n = 9$).	DDA LC-MS/MS	Differential abundance of FASN and 14-3-3ε reveal potential pathways of progression to metastatic melanoma.
2015[47]	Fresh tissue	Primary melanoma ($n = 25$) and lymph node metastasis ($n = 5$).	MALDI-TOF MS	MDA-9 and GRP78 as potential markers for progression to metastatic melanoma.
2015[69]	Fresh tissue	Uveal primary ($n = 7$) and metastatic melanoma ($n = 8$).	iTRAQ labelled DDA LC-MS/MS	Collagen α3 (VI) and HSPβ1 are potential markers of uveal metastasis in mice.
2014[16]	Cell lines	Melanocytes, RGP (WM35, WM1552C), VGP (WM39, WM793B) and metastatic melanoma (1205Lu).	DDA LC-MS/MS	Identification of 4758 proteins with over 200 differentially expressed proteins. Six proteins were identified as potential markers of metastatic potential (vimentin, nestin, fibronectin, annexin A1, dipeptidyl peptidase IV and histone H2A1B).
	FFPE	Metastatic melanoma ($n = 40$).		
2013[50]	FFPE	Benign naevi ($n = 25$), primary ($n = 12$) and metastatic melanoma ($n = 24$).	DDA-LC-MS/MS	A total of 1528 proteins identified with 171 differential proteins between tumor types of FFPE samples that could help diagnosis, prognosis and treatment of melanoma.
2012[52]	Derived cells	Normal ($n = 18$) and dysplastic naevi ($n = 18$).	MALDI-MS/MS	Identification of 16 differentially expressed proteins. Differential proteins were associated with cellular metabolism, detoxification and cytoskeletal organisation processes. These are indicative of dysregulation of cellular process (metabolism and protein synthesis) and increased oxidative stress demonstrating link to the development of melanoma through oxidative DNA damage.
2011[70]	Fresh frozen	Control ($n = 17$) and lymph node metastasis ($n = 69$).	MALDI Imaging Mass Spectrometry	Peak detection of 155 protein features of which 57 peaks with twofold intensity difference between control and melanoma. Protein Signature (e.g. S100A6, cytochrome c, histone H4) to use in the diagnosis and prognosis of stage III melanoma metastasis.
2009[71]	Serum	Control ($n = 19$), Melanoma in situ ($n = 4$), malignant melanoma ($n = 27$).	MALDI-TOF MS	Serum biomarkers (Transthyretin, angiotensinogen and vitamin D binding protein) associated with melanoma and its progression.
2006[72]	Derived cells	Uveal primary melanoma (Mel270) and liver metastatic melanoma ($n = 1$; OMM 1.3, -1.5).	MADLI- MS/MS	Identification of 24 differentially expressed proteins between primary uveal tumor cell line and two metastatic cell lines.
2003[73]	Cell lines	Melanocyte (71B, 71T, 78B, 78T), RGP (WM3208, SBCL2, WM35, WM1789, WM1552C), VGP (WM75, WM39, WM278, WM115, WM793) and metastatic melanoma (WM164, WM1617, A375, HS294T, WM852, WM1232, LU451, WM239A, 1205LU).	MALDI-TOF MS	Identification of 8 candidate protein spots (including HDGF and nucleophosmin B23) as potential markers involved in the progression of melanoma.

Altogether, the advantages of DIA allow for the discovery of potentially novel proteins and important mechanistic pathways involved in disease phenotypes. For instance, Betancourt and colleagues (2021) identified 33 novel proteins from metastatic melanoma patients.[61] Additionally, the reproducibility gained with DIA could prove beneficial in the development of highly confident biomarker signatures, thus improving current classification systems for the diagnosis of disease.[46] Hence, there is an opportunity to apply DIA methodology to investigate melanoma progression in pigmented lesions and identify potential biomarker signatures that could facilitate improvements in early melanoma diagnosis.

5 | NON-INVASIVE DIAGNOSTIC TECHNIQUES

Currently, only imaging is utilised in the clinical setting as a non-invasive diagnostic technique aimed to improve diagnosis and reduce the number needed to biopsy. These techniques include dermoscopy, digital dermoscopy, totally body photography (TBP) and in vivo reflectance confocal microscopy. Additionally, computer-assisted devices (CAD) or clinical diagnosis support are an additional autonomous diagnosis system that can be used alongside dermoscopy and other imaging devices to help classify and detect early melanoma.[74] CAD uses machine learning or a set of patterns to process data.[75] These technologies aim to limit subjectivity in diagnosis and yet their use is not standardised, they still rely on clear guidelines and training for appropriate use. Young and colleagues (2021) recently reviewed the use of these non-invasive technologies and their current potential in the clinical setting[76]; overall, there was an improvement in sensitivity from 76% to 92% (fixed specificity of 80%) with the use of dermoscopy.[77] However, systems like TBP and sequential digital dermoscopy would benefit from targeted usage as excisions have increased potentially conflicting with normal physiological development of naevi in younger persons.[78] Similarly, machine learning is gaining popularity but on the contrary CAD system has not been readily adopted due to insufficient data. Thus, current limitations need to be addressed, and further validation of many of these techniques is still warranted.

Another potential non-invasive alternative utilises the external layer of the epidermis, the stratum corneum. Using an adhesive patch, it is possible to collect cells from the stratum corneum for biological analysis. A study conducted on over 500 pigmented lesions using a combination of adhesive tape and a two-gene (preferentially expressed antigen in melanoma or PRAME and LINC00518 or LINC) classifier was able to diagnose lesions as melanoma or benign.[79] However, there are concerns that melanomas could be missed (1 in 11).[80] In response, Siegel and Hornberger (2019) acknowledge the difficulties of early melanoma diagnosis, especially pertaining to the intermediate lesions; however, using negative predicted value (NPV) which is the likelihood of negative test result being true, the Pigmented Lesion Assay (PLA) method performed better at 99% than traditional histopathology (83%) in reported studies.[79,81] PLA combined two-gene classifier use is readily commercialised, but uptake in the clinical setting has been slow.[18] However, this technology has significant potential in reducing cost, number need to biopsy and increasing access to those that cannot afford numerous procedures and imaging technologies. With all this in mind, the use of an adhesive tape or tape-stripping has been demonstrated to be suitable for proteomic profiling of keratinocytic carcinomas.[82] It would be interesting to see whether the use of adhesive tape-stripping combined with proteomic technologies could be applied similarly in melanoma and benign pigmented lesions. This non-invasive method would allow for genomic and proteomic investigations into pigmented lesions without disturbing histological specimens as well as potentially provide increased accuracy in determining malignant lesions if this were to be developed as a diagnostic technique.

6 | CONCLUSIONS

Clinical and histopathological inspection of melanoma is the most utilised method of diagnosis for melanoma. However, due to the heterogeneous nature of melanoma and its precursors, there are many challenges. The use of genomics has uncovered some molecular pathways and mechanisms involved in melanoma progression. Alternatively, proteomics could provide relevant and novel proteins involved in melanoma progression pathways and help in further classifying these lesions to facilitate the appropriate diagnosis and stratification. The innovation of new non-invasive technologies could help improve current sensitivity and specificity of melanoma diagnosis and reduce the burden of excessive surgery in patients with naevi and other pigmented lesions. Genomics and proteomics on tape-stripping alone or combined with imaging would be a promising avenue in the diagnosis of melanoma.

AUTHOR CONTRIBUTIONS

R.T conducted the review of the literature and involved in writing—original draft preparation. A.A and P.F.P involved in supervision, conceptualisation and writing—reviewing and editing. G.M.P involved in writing—reviewing and editing. M.A and G.J.M involved in supervision and writing—reviewing and editing.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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