Proteomic Profiling of Cutaneous Melanoma Explains the Aggressiveness of Distant Organ Metastasis

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introduction

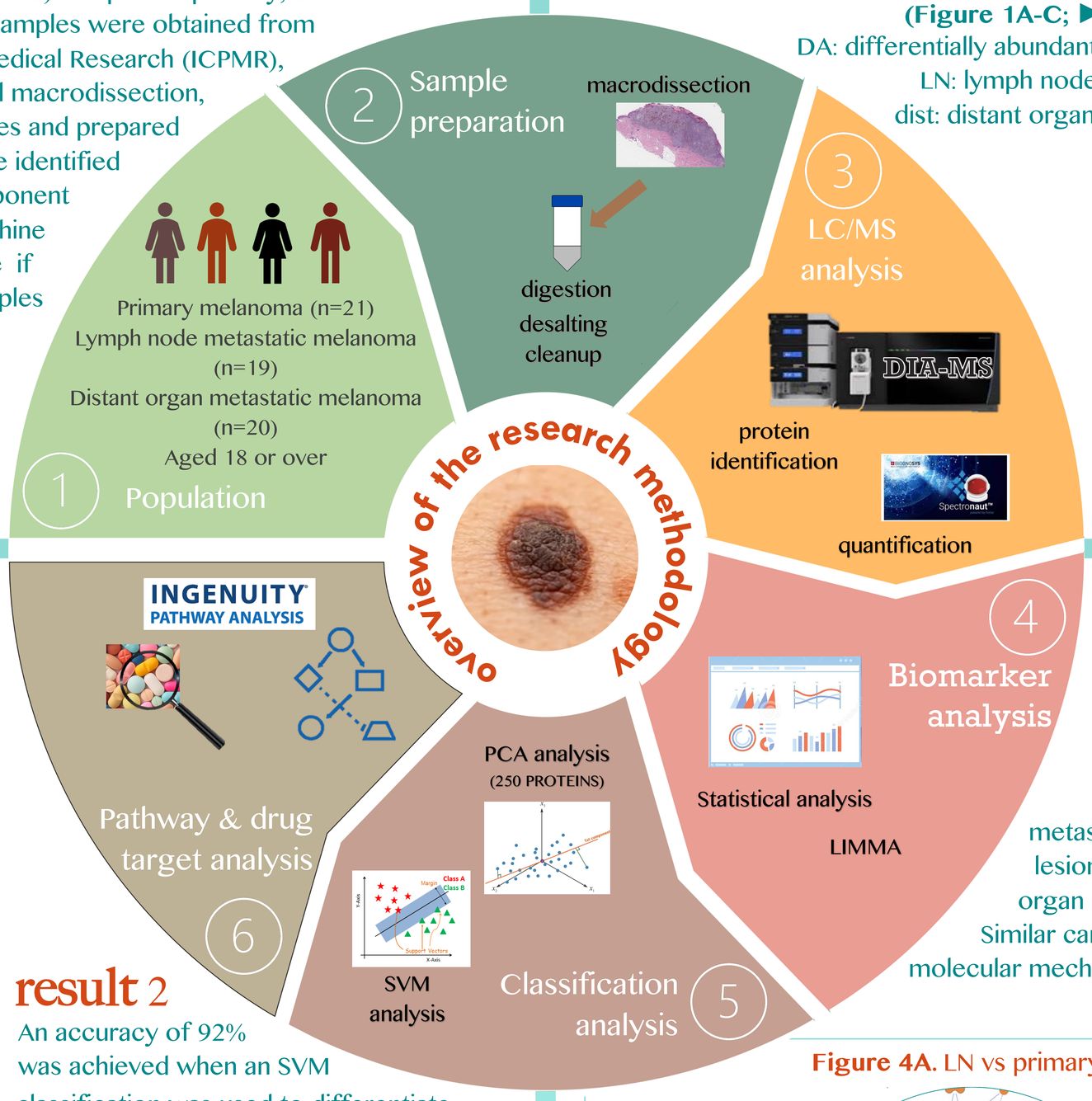
Melanoma remains one of the most aggressive skin tumours. If left untreated, primary melanomas may metastasise to regional lymph nodes (LN) or distant organs. The 5-year relative survival rate reduces from 99.5% in patients with localised melanoma to 70.6% for LN metastatic melanoma and 31.9% when the tumour has metastasised to distant body organs. Therefore, further studies are required to resolve the molecular landscape of metastatic melanomas and deliver impact in novel therapeutic intervention.

objective

To decipher the proteome landscape of primary, LN metastatic and distant organ metastatic melanoma for the identification of protein biomarkers and therapeutic targets with potential clinical significance.

methods

Data-independent acquisition mass spectrometry (DIA-MS) technique was used to investigate the proteomic changes in 60 formalin-fixed and paraffin-embedded (FFPE) samples of primary, LN and distant organ metastatic melanomas. Archival samples were obtained from the Institute of Clinical Pathology and Medical Research (ICPMR), Westmead, repository. Following manual macrodissection, proteins were extracted from the samples and prepared for MS analysis. Protein biomarkers were identified using LIMMA R package. Principal component analysis (PCA) and support vector machine (SVM) analysis were performed to see if proteomic changes can classify the samples based on their metastatic status. Finally, the Ingenuity Pathway Analysis (IPA) bioinformatics tool was used to identify disrupted biological functions and potential therapeutic targets. ▶



result 1

A total of 4,631 proteins were identified, of which 72 and 453 were significantly changed in LN and distant organ metastatic melanomas versus the primary lesions (adj. p-value <0.05).

An increase in proteins such as SLC9A3R1, CD20 and GRB2 and a decrease in CST6, SERPINB5 and ARG1 were associated with regional LN metastasis. In contrast, increased metastatic activities in distant organ metastatic melanomas were related to higher levels of CEACAM1, MC1R, AKT1 and MMP3-9 and decreased levels of CDKN2A, SDC1 and SDC4 proteins.

(Figure 1A-C; ▶
DA: differentially abundant;
LN: lymph node;
dist: distant organ)

Fig 1A: Overall protein identification

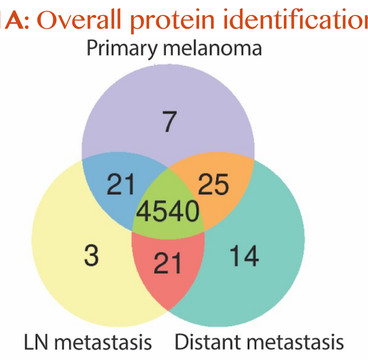


Fig 1B: DA proteins; LN vs primary

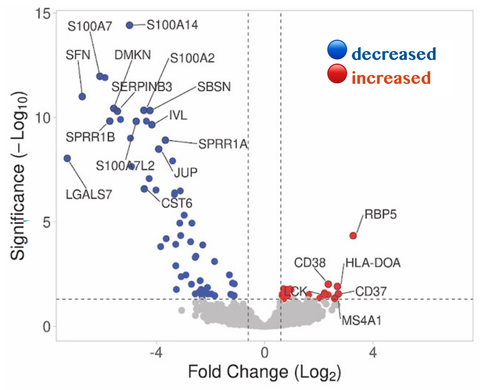


Fig 1C: DA proteins; dist vs primary

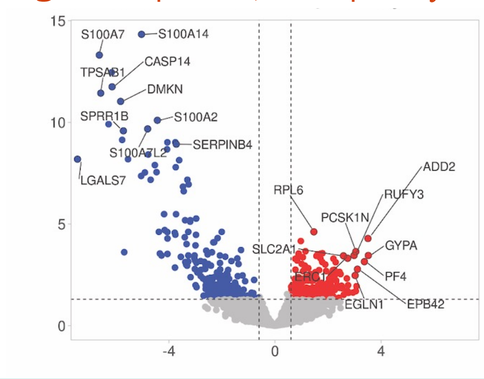


Figure 2A. SVM: LN vs primary

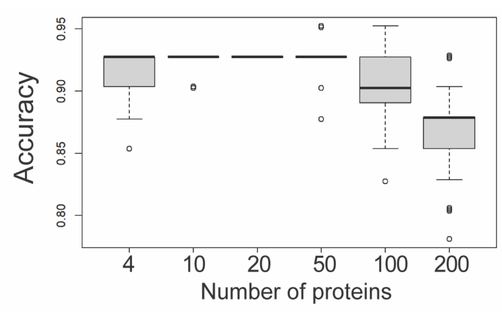


Figure 2B. SVM: dist vs primary

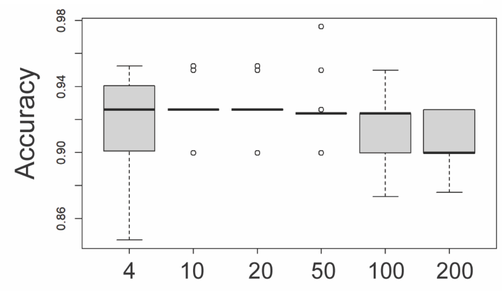
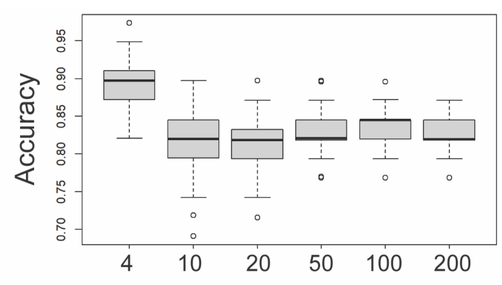


Figure 2C. SVM: dist vs LN



result 2

An accuracy of 92% was achieved when an SVM classification was used to differentiate LN and distant organ metastatic melanomas from the primary lesions. A high accuracy when using the 4-100 most significantly changed proteins indicated that a high-performing biomarker could be constructed with few proteins. A 90% classification accuracy between LN and distant metastatic melanoma was seen using top 4 significantly changed proteins.

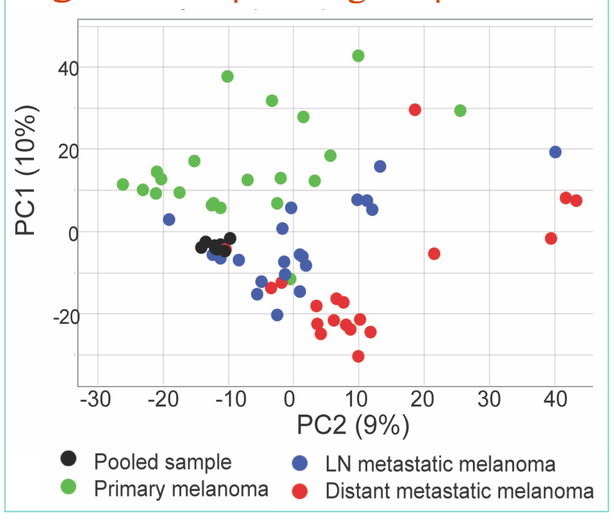
◀ **(Figure 2A-C)**

result 3

When PCA analysis was performed using the 250 proteins with the largest variance, the second PC grouped the samples into primary, LN and distant organ metastatic groups. Also, pooled samples were grouped close to each other, signifying the validity of proteomic analysis. Pooled sample was prepared by adding equal amount of protein from each sample.

(Figure 3) ▶

Figure 3. PCA plot using 250 proteins



result 4

The IPA analysis using differentially abundant proteins revealed that an interplay between complex but distinct networks of proteins is linked with increased invasion and metastasis, and decreased apoptosis in the LN and distant organ metastatic melanomas compared to the primary lesions. This suggests that while LN and distant organ metastatic melanomas present relatively Similar carcinogenic behaviour, but the proteins and molecular mechanisms behind each metastasis are distinct.

▼ (Figure 4A-B)

Figure 4A. LN vs primary

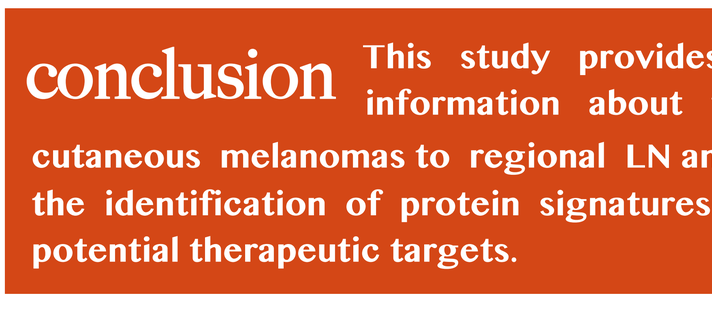
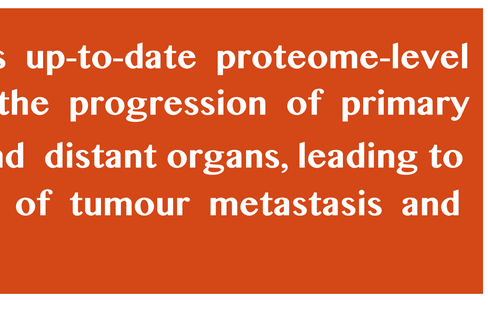


Figure 4B. Distant vs primary



result 5

IPA drug target profiling analysis identified over 10 proteins with significantly increased abundance in the distant organ metastatic melanoma (versus primary lesions) that, subject to further analysis, could be targeted by one or more available medications.

◀ **(Table 1)**

Drug Target	Drug(s)
solute carrier family 2 member 1	canakinumab metformin
solute carrier family 16 member 1	AZD-3965
carbonic anhydrase 1	benzthiazide, ethoxyzolamide
AP2 associated kinase 1	LP-935509, SM1-71
carnitine palmitoyltransferase 1A	perhexiline
tubulin beta 4A class IVa	docetaxel, 5-fluorouracil
solute carrier family 1 member 3	riluzole
protein kinase N2	fasudil
carbonic anhydrase 2	benzthiazide, ethoxyzolamide
thromboxane A synthase 1	ridogrel, dazmegrel

conclusion

This study provides up-to-date proteome-level information about the progression of primary cutaneous melanomas to regional LN and distant organs, leading to the identification of protein signatures of tumour metastasis and potential therapeutic targets.

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