Shallow whole-genome sequencing for assessment of copy number changes in atypical melanocytic neoplasms with adverse outcome

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Introduction

- Dichotomous classification (nevus vs. melanoma) of melanocytic lesions can be difficult.
- Such ambiguity is reflected in descriptive terms as “atypical melanocytic proliferation AMP”, “superficial atypical melanocytic proliferation of uncertain significance (SAMPUS)”, “intraepidermal atypical melanocytic proliferation of uncertain significance (IAMPUS)” and “melanocytic tumor of uncertain malignant potential (MELTUMP)”.[1]
- We previously described hotspot *TERT* promoter (*TERTp*) mutations in approximately 50% of AMP with adverse melanoma specific outcome.[2]
- In the current study we aimed to determine the landscape of copy number changes by applying shallow whole-genome sequencing (sWGS) to a subset of cases.

Methods

- Within the multicentre database of Greater Vancouver, cases between 01/2003 to 12/2018 were identified to allow at least 4 years of clinical follow up.[2] Adverse outcome was defined as subsequent melanoma diagnosis at initial site, or diagnosis of metastatic melanoma in absence of patient history of invasive melanoma at any other site.
- Immunohistochemistry (IHC) and *CDKN2A/B* FISH were performed using standard protocols.
- For sWGS, DNA libraries were constructed using the ThruPlex DNA-seq kit (Takara), and sequencing performed on an an Illumina NovaSeq machine. Raw reads were processed using an in-house Nextflow pipeline.[3] Copy number profile quality was assessed using Utanos, an in-house toolkit for the analysis of sWGS data.[4]

Results – Study Cohort

Case no.	Age (y)	Sex	Site	Original diagnosis	Histology/ WHO classification	Mutational events	Outcome (stage)	Time to adverse outcome (months)
1	44	F	Left leg	Atypical compound melanocytic lesion with spitzoid features	Pigmented epithelioid melanocytoma	*TERTp* mut	Regional LN metastasis (N1)	8.5
2	57	F	Flank	Intradermal melanocytic nevus with atypical spitzoid features	BAP1-inactivated melanocytoma	*BAP1* deletion	Metastatic melanoma (M1c)	35.4
3	35	M	Neck	Combined conventional and epithelioid blue nevus	Pigmented epithelioid melanocytoma	*TERTp* mut	Regional LN metastasis (N2)	5.8
4	38	M	Neck	Deep penetrating nevus	Wnt-activated melanocytoma	n.a.	Subcutaneous metastasis (M1a)	12.5

Table 1: Clinical characteristics of the investigated study cohort. After review of residual DNA availability from seven cases in which *TERT* promoter mutation status was determined,[2] amplification was successfully performed in a total of four cases, which were subjected to sWGS. Age in years (y), female (f), male (m), not otherwise specified (NOS), lymph node (LN), *TERT* promoter mutated (*TERTp* mut).

Results – Case Analysis

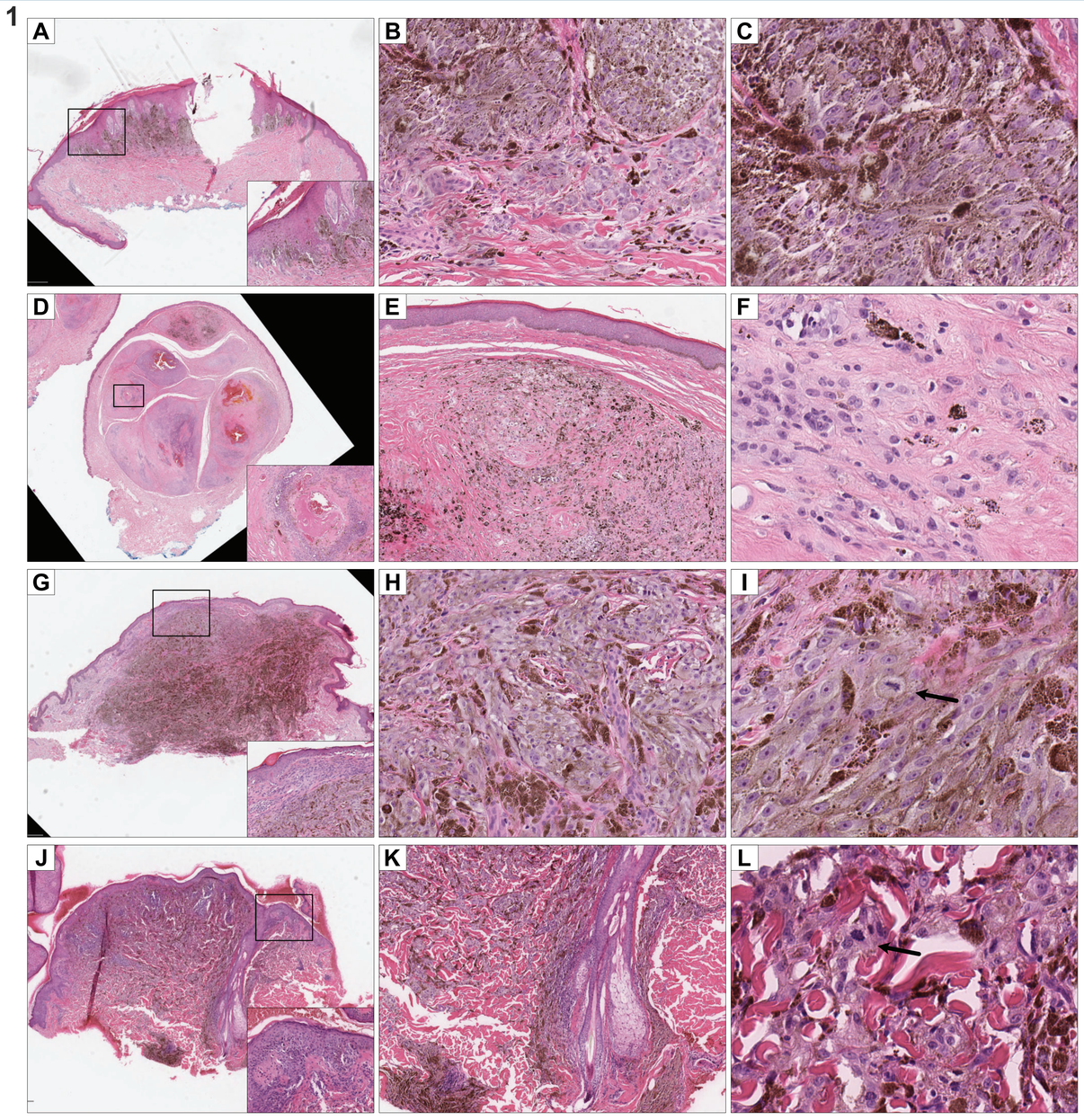

Figure 1: Histomorphological characterization of the cases investigated in the study. (A-C) case 1, (D-F) case 2, (G-I) case 3, (J-L) case 4). H&E staining, original magnification x 5 (D), x20 (A; G; J), x50 (E, K), x100 (inset A; inset, D; inset J), x200 (B; inset, G; H; inset J), x 400 (C; F; I; L).

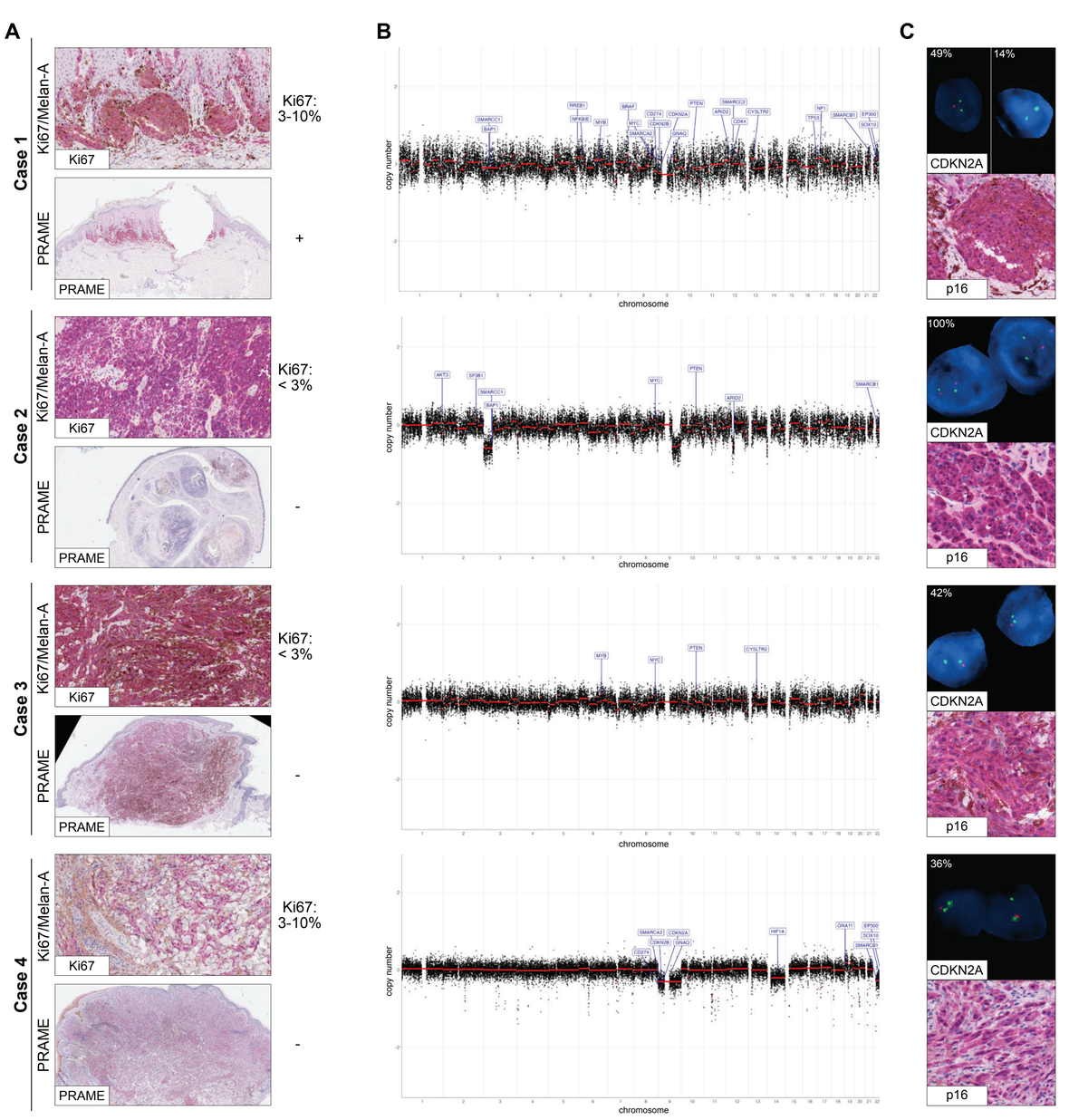

Figure 2: Molecular analysis of the cases. (A) Representative images of Ki67/Melan-A staining (x100) and PRAME (x20). (B) Copy number (CN) plots, normalized for individual case reads. A $\log_2$ ratio of 0 represents an unaltered CN state. CN losses defined as $\log_2$ ratio < -0.1, CN gains as a $\log_2$ ratio > 0.1. (C) *CDKN2A*-FISH and p16 IHC (x200).

Conclusion

- sWGS revealed heterogeneous CN variation patterns among AMP, suggesting a potential role for CN variations in the biological diversity and malignant potential of these tumors.
- sWGS detected *CDKN2A/B* CN variations, including subclonal events such as heterozygous deletions, which may play a role in biology of AMP with adverse clinical outcome.

Discussion and Outlook

- sWGS has potential as an ancillary rapid and cost-effective method for evaluation of melanocytic lesions for which little study material is available, which is often a limiting factor.
- Molecular diagnostics for classification of AMPs are inhomogeneous regarding availability and interpretation to date, rendering further studies neccessary.

Literature

1) WHO Classification of Tumours Editorial Board. Skin tumours. Lyon (France): International Agency for Research on Cancer; forthcoming. (WHO classification of tumours series, 5th ed.; vol. 12). https://publications.iarc.fr. 2) Huber R, Lee J, Borretta L et al. TERT promoter mutations in atypical melanocytic lesions: A series of seven cases with adverse melanoma-specific outcome. Hum Pathol. 2024 Feb;144:34-39. 3) Di Tommaso P, Chatzou M, Floden EW, Barja PP, Palumbo E, Notredame C. Nextflow enables reproducible computational workflows. Nat Biotechnol. 2017 Apr 11;35(4):316-319. 4) Jamieson A, Sobral de Barros J, Cochrane DR et al, Targeted and Shallow Whole-Genome Sequencing Identifies Therapeutic Opportunities in p53abn Endometrial Cancers. Clin Cancer Res. 2024 Jun 3;30(11):2461-2474.

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