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Consensus of Melanoma Gene Expression Subtypes Converges on Biological Entities

Journal of Investigative Dermatology (2016) **136,** 2502–2505; doi:10.1016/j.jid.2016.05.119

TO THE EDITOR

Identification of recurrent mutation in the *BRAF* oncogene in melanoma has led to the development of highly selective kinase inhibitors (Larkin et al., 2014). Although dramatic treatment responses are initially observed, responses are rarely durable. The mutational classification based on *BRAF, NRAS,* and *NF1* mutations that has been established, however, is nonoverlapping with classification derived from gene expression profiling (The Cancer Genome Atlas Network [TCGA], 2015; Jönsson et al., 2010). In 2010, we reported four expression-based melanoma subtypes (the Lund subtypes): the high-immune, normal-like, microphthalmia-associated transcription factor (MITF)-high pigmentation, and MITF-low proliferative groups (Jönsson et al., 2010). The high-immune group was distinguished by elevated expression of immune genes, the normal-like group by genes expressed in surrounding normal cells; the MITF-high pigmentation and the MITF-low proliferative groups displayed increased expression of cell-cycle genes, and the MITF-low proliferative group had decreased expression of melanocyte differentiation genes. These subtypes were derived in stage IV tumors (Jönsson et al., 2010), but have since been firmly established in primary tumors (Harbst et al., 2012; Nsengimana et al., 2015) and stage III tumors (Cirenajwis et al., 2015). In 2015, a TCGA landmark study reported three melanoma gene expression subtypes (TCGA, 2015). The immune group was characterized by increased expression of immune genes; the keratin group was characterized by overexpression of keratin, pigmentation and epithelial genes; and the MITF-low group displayed decreased expression of melanocyte differentiation genes and activation of genes involved in nervous system development. However, it is not clear how the biological processes underlying the classification schemes relate to each other. Here, we compare bioinformatically derived biological pathways between the TCGA and Lund subtypes to search for evidence that both classification schemes identify similar biological entities, which may prove to be relevant in patient care.

To compare the TCGA and Lund classification schemes, we first investigated the two reported gene sets. Of the 1,500 TCGA genes used for subtype discovery, only 34 overlapped with the 486 Lund genes (Figure 1a). Despite this limited overlap, gene ontology-term analysis showed similar biological processes enriched (false discovery rate < 0.01) in the two gene sets, including immunological processes (e.g., immune response, TCGA; response to wounding, Lund), melanocyte development (e.g., epidermis development, TCGA; pigmentation, Lund), and cell adhesion (e.g., cellular adhesion, TCGA and Lund). In addition, neuronal development processes were enriched in the TCGA gene set alone (see Supplementary Figure S1 online). Next, we investigated whether absolute expression levels of the TCGA and Lund gene sets differed (see Supplementary Materials online). Most TCGA genes were expressed at remarkably low levels, whereas the Lund genes were drawn from the entire expression range ($P = 1 \times 10^{-68}$, Figure 1A). This suggests that the limited gene overlap between the TCGA and Lund classification schemes was caused by the technical relationship of intensity and variance (see Supplementary Figure S1), not by biological divergence. Collectively, although the gene sets were obtained from different expression ranges, they represent similar biological processes.

Next, we compared actual sample classifications between the TCGA and Lund schemes. First, we classified the 329 TCGA samples (TCGA, 2016) (Table 1) according to the Lund subtypes (Cirenajwis et al., 2015). An extensive overlap between TCGA and Lund classification schemes was observed (Figure 1b). Specifically, the TCGA immune group consisted of the Lund high-immune group (88%) plus 55% of MITF-high pigmentation group samples. The TCGA keratin group consisted predominantly of the Lund normal-like and MITF-high pigmentation groups, whereas the TCGA MITF-low group contained 76% of the Lund MITF-low proliferative tumors. Next, we applied the TCGA subtypes to the published Bergen (Jönsson et al., 2010) (GSE33153), Lund (Cirenajwis et al., 2015) (GSE65904) and Leeds (Nsengimana et al., 2015) (E-MTAB-4725) datasets, which have pre-existing Lund subtype classifications. The fraction of subtypes differed between datasets, according to specimen site. The primary cohort of Leeds and the primary samples of the other datasets displayed small fractions of the high-immune and immune subtypes and high fractions of normal-like and keratin samples, whereas regional metastases contained few normal-like and keratin samples (see Supplementary Figure S2 online). Association between classification schemes and age at diagnosis and sex did not show consistent significance across the four datasets. The Lund and TCGA subtypes were

Abbreviations: MITF, microphthalmia-associated transcription factor; TCGA, The Cancer Genome Atlas network

Accepted manuscript published online 23 June 2016; corrected proof published online 5 August 2016

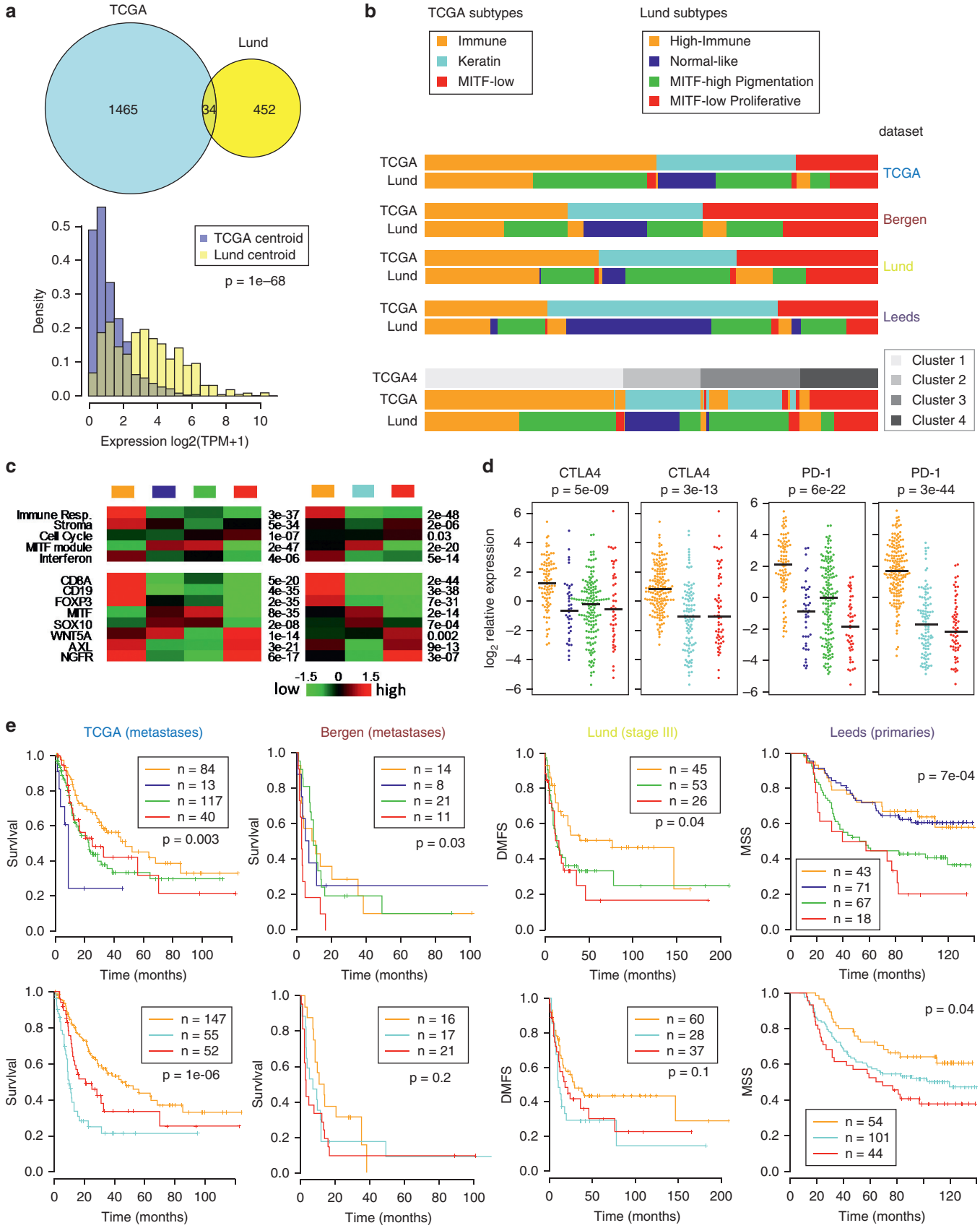

Figure 1. Consensus of gene expression classification of melanoma. (**a**) Upper panel: number of genes defined in the TCGA and Lund gene sets, displayed by Venn diagram. Lower panel: Absolute gene expression of the TCGA and Lund gene sets. *P*-value from *t* test. (**b**) Overlap of TCGA and Lund gene expression subtypes in four datasets. TCGA4 indicates clusters of TCGA samples using consensus hierarchical clustering with four groups. (**c**) Heatmap of (upper panel) the relative expression of gene modules and (lower panel) genes of biological interest (proliferative: *MITF, SOX10;* invasive: *WNT5A;*

Table 1. Datasets

	TCGA	Bergen	Lund	Leeds
Reference	(TCGA, 2015)	(Jönsson et al., 2010)	(Cirenajwis et al., 2015)	(Nsengimana et al., 2015)
n	329	57	214	199
Age in years, mean (range)	60 (20–90)	64 (25–86)	64 (22–91)	58 (20–79)
Sex, n (%)				
Female	111 (38)	26 (46)	89 (42)	102 (51)
Male	180 (62)	31 (54)	124 (58)	97 (49)
Specimen site, n (%)				
Primary	64 (19)	0	16 (8)	199 (100)
Regional metastasis	228 (70)	3 (5)	128 (60)	0
Distant metastasis	35 (11)	54 (95)	59 (28)	0
Not available	2 (0)	0 (0)	11 (4)	0 (0)
TCGA subtype, n (%)				
Immune	168 (51)	18 (32)	82 (38)	54 (27)
Keratin	102 (31)	17 (30)	65 (30)	101 (51)
MITF-low	59 (18)	22 (39)	67 (31)	44 (22)
Lund subtype, n (%)				
High-immune	89 (27)	15 (26)	73 (34)	43 (22)
Normal-like	42 (13)	8 (14)	12 (6)	71 (36)
MITF-high pigmentation	152 (46)	22 (39)	90 (42)	67 (34)
MITF-low proliferative	46 (14)	12 (21)	39 (18)	18 (9)

Abbreviations: MITF, microphthalmia-associated transcription factor; TCGA, The Cancer Genome Atlas network.

interrelated in a similar way in all three datasets, as already observed in the TCGA dataset (Figure 1b), validating the overlap of the two subtyping schemes. Because the TCGA subtypes consist of three groups and the Lund subtypes of four groups, we derived four consensus clusters from the TCGA data for straightforward comparison (see Supplementary Figure S3 online). A cluster appeared that included 95% of the samples of the normal-like group (Figure 1b). Overall, these results show a high consensus between the TCGA and Lund classification schemes.

Next, we investigated how key melanoma genes and transcriptional programs were expressed across the subtypes to show the biological basis underlying the classification schemes. We recently described gene modules, capturing major expression directions in melanoma (Cirenajwis et al., 2015). The MITF-module was up-regulated in the keratin, normal-like, and MITF-high pigmentation groups and down-regulated in the MITF-low and MITF-low proliferative groups (Figure 1c). In addition, markers of melanoma cell states and neural crest progenitors were differentially expressed across subtypes (Figure 1c). Markers of cytotoxic T cells (*CD8A*), B cells (*CD19*), and regulatory T cells (*FOXP3*), as well as the immune response module, were up-regulated in the immune and high-immune groups. Targets of immune checkpoint blockade agents, *CTLA4* and *PD-1*, were also highly expressed in the immune and high-immune groups (Figure 1d). Overall, key melanoma genes displayed analogous expression in the immune and high-immune groups; keratin, normal-like, and MITF-high pigmentation groups, and MITF-low and MITF-low proliferative groups. We therefore argue that these sets of subtypes have distinct biological backgrounds.

The immune groups (immune and high-immune) had favorable survival rates compared with the remaining groups in patients with metastatic samples from TCGA, Bergen, and Lund and in the primary Leeds cohort (Figure 1e), confirming previous reports. Most patients were recruited before systemic treatment; thus, the treatment-predictive significance of the subtypes could not be assessed. However, there is emerging evidence that expression of immune genes may predict response to immune checkpoint blockade (Van Allen et al., 2015). Further studies are needed to determine the treatment predictive value of gene expression subtypes.

Collectively, melanoma gene expression patterns are determined along the trajectories of melanocyte differentiation and mitotic genes, and genes expressed in surrounding/infiltrating immune cells and stroma. Single genes may deviate from these patterns; however, we show that genome-wide expression analysis converges on fundamental melanoma entities, as represented by the TCGA and Lund subtypes.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

The study was supported by the Swedish Cancer Society, The Swedish Research Council, BioCARE, Berta Kamprad Foundation, The Gunnar Nilsson Cancer Foundation, The Gustav Vth Jubilee Foundation, and Governmental Support for Medical Research (ALF). The Leeds data were built using funding from Cancer Research UK Programme grant C588/A19167 and Project Grant C8216/A6129. The research leading to these results has received funding from the European Community's Horizon 2020 Framework Programme for Research and Innovation (H2020-MSCA-ITN-2014) under Grant Agreement n°247634.

AUTHOR CONTRIBUTIONS

ML and GJ conceived and designed the study and drafted the manuscript. JS and JN performed additional analyses. JN-B and GJ supervised the study. All authors read and approved the manuscript.

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neural crest progenitor: *NGFR*), averaged per subtype. *P*-value from analysis of variance. (**d**) Relative gene expression of *CTLA4* and *PD1* across subtypes. *P*-value from analysis of variance. (**e**) Kaplan-Meier plots of disease outcome per subtype. *P*-value from Cox regression. TCGA cohort consists of patients with tumor samples from regional lymph node, regional skin, or soft tissue and distant metastasis, Bergen cohort of advanced local and distant metastases, and Lund cohort of patients with stage III tumors. Normal-like group containing a single sample was omitted; Leeds melanoma cohort includes primary tumors sampled from formalin-fixed blocks. DMFS, distant metastasis-free survival; MITF, microphthalmia-associated transcription factor; MSS, melanoma-specific survival; TCGA, The Cancer Genome Atlas network.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http:/dx.doi.org/10.1016/j.jid.2016.05.119.

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