Standard melanoma-associated markers do not identify the MM127 metastatic melanoma cell line

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Dear Editor,

Melanoma cell lines are an essential tool for melanoma research (Yu et al., 2015). Melanoma cells are routinely identified using antigens encoded by genes that are commonly expressed in melanocytes and melanoma cell lines (Urosevic et al., 2005). Common markers include: S100; HMB-45; and Melan-A (Viray et al., 2013). Our study explores the expression of these three markers in four different human melanoma cell lines: WM35; WM793; SK-MEL-28; and MM127. All melanoma cell lines we use are validated using short tandem repeat (STR) profiling (Cell Bank, Australia. January 2015). The alleles obtained from STR profiling are analysed using the DMSZ database (http://www.dsmz.de/fp/cgi-bin/str.html) to give the closest match to each cell line we consider. Results for the metastatic melanoma cell line, MM127, are not as expected since there is no match identified using the MM127 alleles (Figure 1A). We also examine the expression of S100; HMB-45 and Melan-A at both the mRNA and the protein level by performing immunofluorescence, Western blotting and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) assays. Again, our results for the metastatic melanoma cell line, MM127, are not as expected. This cell line is not identifiable using any of the three markers. Since the MM127 cell line does not express any of the standard melanoma-associated markers, we suggest that it would be

difficult to perform further melanoma-related experiments using this cell line, and that this cell line should be used with care.

Since melanoma cells are known to express a diverse range of surface antigens, previous studies often use multiple markers for reliable identification (Behren et al., 2013). We use immunofluorescence on fixed cell preparations for S100, HMB-45 and Melan-A. The cell nuclei, f-actin and three standard melanoma markers are highlighted (Figure 1B). S100 is localised to the nucleus and cytoplasm, and is observed in WM35, WM793 and SK-MEL-28 cells. HMB-45 is detected in the cytoplasm of WM35, WM793 and SK-MEL-28 cells (Figure 1B). WM35 and SK-MEL-28 cells show Melan-A staining, whereas it is absent from WM793 cells. Interestingly, MM127 is the only melanoma cell line we examine that is negative for all three markers in our immunofluorescence investigations. To confirm these results, we also validate our immunofluorescence studies with Western blotting analysis.

The expression of S100 (10kDa), HMB-45 (27 and 100kDa), and Melan-A (18kDa) proteins in the melanoma cell lines: WM35; WM793; SK-MEL-28; and MM127, are analysed using Western blots. HMB-45 is detected as two bands, which is consistent with previous results (Kawakami et al., 2008). The cell lines WM793 and MM127 are negative for Melan-A and HMB-45 (Figure 1C). The absence of HMB-45 in WM793 cells in the Western blots does not coincide with our immunofluorescence results (Figure 1B). This is an interesting result that has not been reported in previous studies. Discrepancies in protein expression among individual melanoma cells has been reported previously (Kim et al., 2013), and we observe a similar variation in the WM793 cells, since some individual WM793 cells are positive for HMB-45 (Figure 1B) while other WM793 cells are negative for HMB-45 (not shown). However, most importantly for our work, the expression of all three proteins is absent from the MM127 cells. These results for the MM127 cell line concur with our immunofluorescence results (Figure 1B) and suggest that the MM127 cell line does not

express the same antigens as the other melanoma cell lines we consider. To provide additional confirmation of our immunofluorescence and Western blot data, we also perform qRT-PCR.

The identification of melanoma cells in a heterogeneous tissue sample is very important (Sheffield et al., 2002), and qRT-PCR can be used to identify a small number of melanoma cells within such a heterogeneous sample (Fleige et al., 2006). In our present study we quantify genes that encode specific proteins associated with melanoma cell lines. We use the HaCaT cell line as a negative control because this cell line does not express any melanoma-associated markers (Figure S1). Our results indicate that the expression of S100 (*S100B)*, Melan-A (*MLANA)* and HMB-45 (*PMEL*) in MM127 is very similar to the negative control (Figure 1D). The qRT-PCR result for S100 in the HaCaT cell line is reported as NA since the gene level is undetectable. To further verify our results, we also examine the expression of two other commonly-used melanoma genes, microphthalmia-associated transcription factor (*MITF*) and tyrosinase (*TYR*) (Sheffield et al., 2002). Again, we find that the expression of *MITF* and *TYR* in the MM127 cells are very similar to the negative control (Figure S2).

Collectively, our findings indicate that standard melanoma-associated markers are not detected in the MM127 cell line. Although the MM127 cell line has been used in several previous studies (Cozzi et al., 2006; Goss et al., 1977; Treolar et al., 2013), we find that this cell line cannot be detected using standard melanoma-associated markers. Therefore, we suggest that other metastatic melanoma cell lines that express S100, HMB-45 or Melan-A, such as the SK-MEL-28 cell line, ought to be used in preference to the MM127 cell line. Beyond our recommendation that the MM127 cell line ought to be used with care, we note that our results are consistent with the idea that MM127 cells are, in fact, not a melanoma cell line at all. Since the MM127 cell line was first discussed in 1979 (Pope et al., 1979), it is possible that the MM127 cells currently available from Cell Bank, which are identical to the

cells used in our current study (Supplementary Material), are somehow different to the cells originally reported in 1979. However, more research is required to provide a definitive characterization of the MM127 cell line, and to determine whether this cell line retains any of the melanoma-associated features that were originally of interest.

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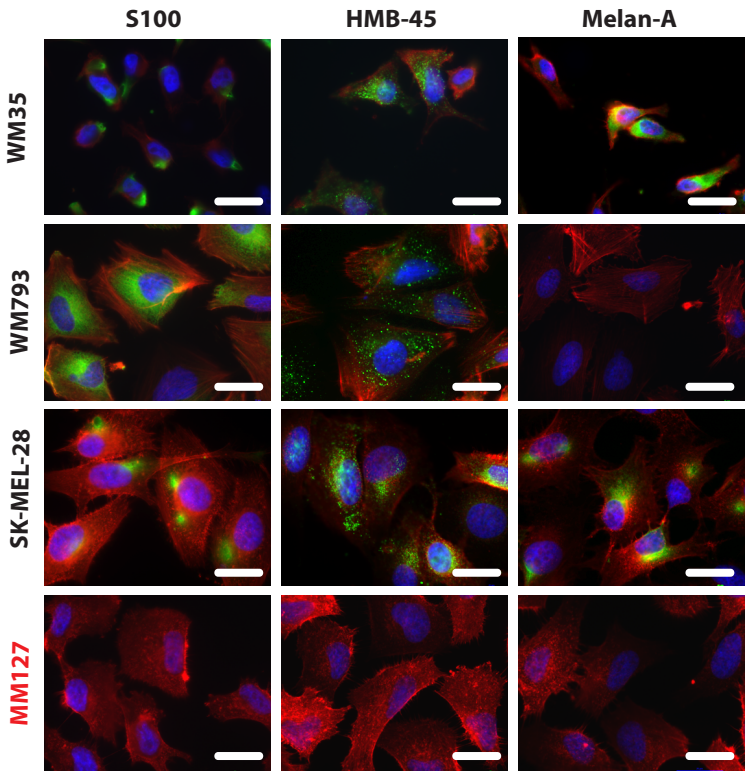
Figure Legend

Figure 1: MM127 cell line does not express three standard melanoma-associated proteins. (A) STR analysis of the MM127 cell line. Upper row shows the allele names. Lower row describes the location of the allele on the chromosome. (B) Immunofluorescence results for four melanoma cell lines: WM35; WM793; SK-MEL-28; and MM127. Cells are fixed in 4% paraformaldehyde and stained for S100 (green), HMB-45 (green) and Melan-A (green). The nucleus (blue) and f-actin (red) are highlighted. Scale bar 25μm. Results for the negative control (HaCaT) are given in the supplementary material. (C) Melanoma (WM35, WM793, SK-MEL-28 and MM127) and negative control (keratinocytes, fibroblasts, melanocyte primary cells and HaCaT cell line) are analysed by Western blotting for S100, HMB-45 and Melan-A. GAPDH is used as loading control and detected at 37kDa. (D) qRT-PCR results are shown as a graph showing the difference between the expression of melanoma-associated genes for the negative control (HaCaT) and various cell lines. Values correspond to $\Delta Ct = Ct$ (*RPL32*) – Ct (*target gene*) ± standard error, (n=3).

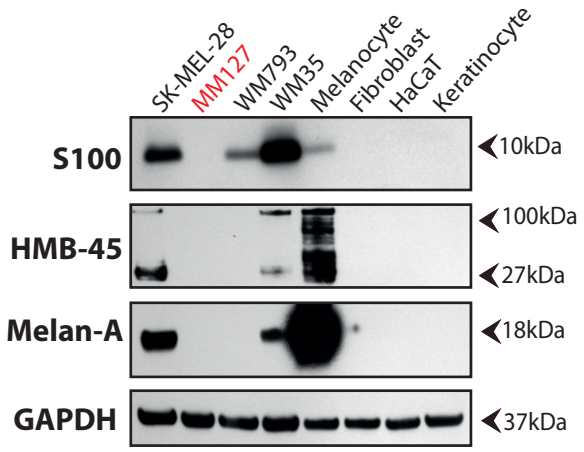
A

D3S1358	TH01	D21S11	D18S51	Penta E	D5S818	D13S317	D7S820	D16S539	CSF1PO	Penta D	Amel	vWA	D8S1179	TPOX	FGA
15,17	8,9	29	15,20	5,10	11,12	12,13	10,11	10,13	10,11	10	X,Y	16,18	14,15	11,12	14,15

B



C



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