Validation of an immunodiagnostic microneedle device for the detection of the melanoma biomarker, S100B

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Introduction

Melanoma is the deadliest form of skin cancer. An ability to detect melanoma early and rapidly could enable effective melanoma screening and improve prognosis. S100B is an intra-tumoural biomarker used in histopathological diagnosis of melanoma. Its serum concentration has been associated with melanoma progression and prognosis [1]. Here, we have validated an immunodiagnostic microneedle device for detecting S100B, with a view to developing a diagnostic tool for early and rapid melanoma detection.

Materials and methods

The device is based on an adapted sandwich enzyme-linked immunosorbent assay (ELISA). Microfabricated polylactic acid microneedles were surface-functionalised with an anti-human S100B capture antibody (clone: 8B10). The microneedles were immersed in 100 μg/mL recombinant human S100B or inserted into a 3-dimensional melanoma (A375) culture for 1–3 h. Captured S100B was detected with a peroxidase-labelled, anti-human S100B detection antibody (clone: 6G1). The chromogenic substrate was o-phenylenediamine. Colour signals were blotted on chromatography paper for visualisation [2]. Since the culture medium contained 15% v/v foetal bovine serum (FBS), to

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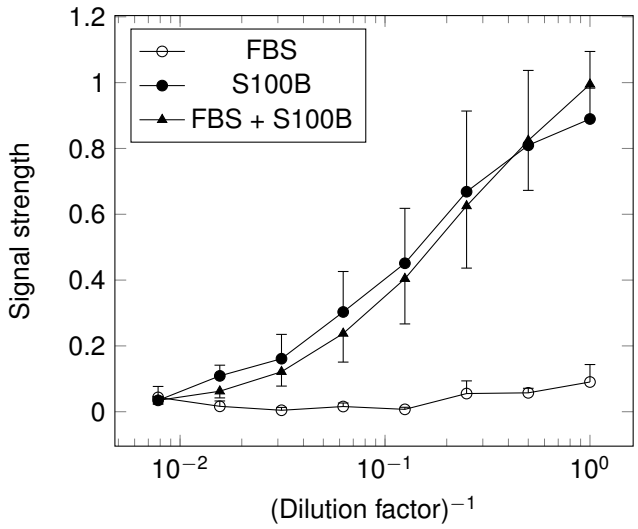

Figure 1: ELISA result showing relationship between signal strength (mean ± standard deviation, $n = 5$ independent experiments) and sample dilution factor, as a surrogate measure of S100B concentration. Signal strength = $(A - A_{\min})/(A_{\max} - A_{\min})$, where A is the absorbance of a given sample, $A_{\max}$ and $A_{\min}$ are the maximum and minimum absorbance observed in the same experiment, respectively. Error bars are staggered for clarity.

preclude cross-reactivity with FBS components (particularly bovine S100B), sequence homology between human and bovine S100B was analysed using the BLASTp tool (v2.8.1; https://blast.ncbi.nlm.nih.gov). Plate-based ELISA was performed on serially diluted culture medium containing 4–500 ng/mL recombinant human S100B, using the same ELISA reagents as the microneedle device. Results were compared with culture medium minus FBS or S100B.

Results and discussion

The microneedle device detected S100B in the recombinant human S100B solution and A375 culture. BLASTp analysis showed that human and bovine S100B proteins were 97% identical. Plate-based ELISA showed an inverse relationship between sample dilution factor and signal strength in all samples containing human S100B, whereas those with FBS minus human S100B flatlined at background levels (Figure 1). Thus, there was no evidence of cross-reactivity between the microneedle device and FBS, confirming the cellular origin of the S100B detected in culture.

Conclusion

The immunodiagnostic device can detect human S100B in a 3-dimensional culture of human melanoma cells in vitro. The device has potential diagnostic applications in

melanoma detection.

References

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