Characterisation of DNA methylation machinery in human skin

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Introduction

- DNA methylation is an epigenetic mark usually associated with gene silencing, commonly referred to as switching genes 'on' or 'off.'
- The methylation state is influenced by 'writer' DNA methyltransferases (DNMTs) and the putative 'eraser' ten-eleven translocases (TETs) (fig 1).
- DNA methylation machinery in the skin remains to be characterised, and may influence appropriate epidermal stratification.

We sought to characterise the epidermal distribution and subcellular localisation of DNA methylation machinery in healthy human skin and in a cellular model of normal epidermal stratification.

Fig. 1 DNA methylation and demethylation pathway.

Methodology

- Skin acquired via The University of Manchester Skin Health Biobank (MSHB) was analysed for DNMT and TET expression by immunohistological techniques.
- Primary keratinocytes grown to confluence were induced to differentiate via addition of 1.8mM CaCl_2 ('calcium switching') and analysed by immunofluorescence and qPCR for DNMT expression.

Methylation machinery is differentially distributed in human skin

- Our staining confirmed the expression pattern of DNMT1 (fig 2a, b) previously reported by Sen et al. (2010) within the basal layer of the epidermis with limited expression in the outer, differentiated layers.
- DNMT3B is expressed ubiquitously across the epidermis in distinct foci overlapping with nuclei (fig 2c, d).
- Intense cytosolic immunoreactivity for DNMT3A of epidermal basal cells with diffuse nuclear staining seen in outer differentiated layers (fig 2e, f).

Fig. 2 DNA methyltransferase protein distribution in adult human abdominal skin. Dashed line represents the dermal-epidermal junction, arrows indicate immunoreactivity.

Calcium switching shows concordant regulation of methylation machinery

- Immunofluorescence analysis of primary human keratinocytes confirms immunoreactivity in the *stratum basale* of human skin (fig 3).
- Calcium switching cells down regulated DNMT1 and DNMT3b expression, while DNMT3a was reduced slightly but was not significant (fig 4).

Fig. 7 Protein expression analysis of DNA methyltransferases in primary human keratinocytes. Immunofluorescence analysis of DNMT1 (a), DNMT3a (b), DNMT3b (c) and TET1 (d) Western blotting analysis of DNMT3a shows ablation of higher molecular weight band upon differentiation (e).

Fig. 6 qPCR analyses for DNA methyltransferases in non-differentiated and Ca^{2+} differentiated primary keratinocytes.

mRNA expression fold-change before (left) and after (right) differentiation. Differentiation was confirmed by upregulation of involucrin transcripts. Genes were normalised to peptidylprolyl isomerase A (PPIA). The bar indicates mean $\pm$ SEM; * One-Sample T-Test: $P < 0.05$; ** One-Sample T-Test: $P < 0.05$.

Conclusion

- DNA methyltransferases show differential distribution and subcellular localisation, suggesting distinct roles in gene activation and repression.
- Regulation of DNMTs within the epidermis may have implications for the reduced epidermal turnover seen in ageing.