Clobetasol-induced quiescence in the vulvar carcinoma cell line, UMSCV-4 can be overcome by repeated removal and re-exposure to this ultrapotent corticosteroid

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INTRODUCTION

Vulvar cancer is rare, mostly afflicting women aged 60 and older [1]. The cancer often preceded by a common vulvar rash, Lichen sclerosis, that is usually treated with the ultra-potent corticosteroid, clobetasol propionate. This treatment may, in turn, be associated with vulvar carcinogenesis. Quiescence, a common characteristic of stem cells, is a reversible state of growth arrest. Our results suggest that clobetasol is causing UMSCV-4 vulvar carcinoma cells to enter quiescence, and may allow them to evade the standard treatments that target rapidly proliferating populations. Furthermore, when these cells are removed from clobetasol and re-exposed, they proliferate at higher levels. This suggests that the initial clobetasol treatment selects for cells that are now unable to enter quiescence when re-exposed to clobetasol.

RESULTS

Cell death assays (trypan blue) and immunofluorescence detection of the proliferation markers Ki67 as well as BrDU incorporation were used to determine rates of proliferation in the clobetasol treated UMSCV-4 cells. In addition, cells were examined for changes in mRNA expression of key markers indicative of entering a state of quiescence. Clobetasol was diluted in 95% ethanol (10⁻⁷ M final concentration) and UMSCV-4 cells were cultured in the presence (+clob) or absence of clobetasol (-clob) for these experiments. UMSCV-4 LT cell populations were generated as described in Figure 1.

The basic procedure for examination of clobetasol effects on UMSCV-4 NT and UMSCV-4 LT cells was as follows:

- EOH 10c: Ethanol treated for 10 days
- Clob10d: Treated with clobetasol for 10 days
- Clob 6d-4d: Treated with clobetasol for 6 days and changed to ethanol medium for 4 days

Cells from each group were subsequently harvested and tested using protocols described in each figure.

CONCLUSIONS

- Clobetasol treatment lead to increased cell death in UMSCV-4 cell subpopulations that were not previously exposed to clobetasol (NT).
- Clobetasol treatment lead to decreased proliferation in UMSCV-4 cell subpopulations that were not previously exposed to Clob (NT). This could be reversed within 4 days if cells were removed from Clob after 6 days treatment.
- UMSCV-4 cells that were treated for 3 months with clobetasol, then removed, and re-exposed (LT) showed similar proliferation rates when treated with clobetasol, ethanol, or clobetasol short term.
- NT cells had upregulation of cell cycle inhibitors, p16 and p21 while downregulation of cyclin D1 after 10 days of clobetasol treatment, indicating a negative change in the proliferation.

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REFERENCES

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