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Induction of Cell Differentiation in Human Cancer Cell Lines by Histone Deacetylase Inhibitors and Sesquiterpene Lactones

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1. We have established a sensitive model to identify inducers of colon cancer cell differentiation by alkaline phosphatase (ALP) induction in the HCT 116 colon cancer cell line and its p53 and p21 (-/-) derivatives.

2. We demonstrate that p21 is essential for induction of differentiation by sodium butyrate as sodium butyrate failed to induce differentiation in p21 (-/-) cells. Our observations that sodium butyrate induced elevated ALP activity in p53 (-/-) cells implicate that induction of differentiation by butyrate was p53-independent.

3. Interestingly, TSA, a commonly used HDAC inhibitor, was unable to induce ALP activity, a marker of cell differentiation, whereas sodium butyrate and TSA induced similar hyperacetylation of histone H4 in HCT 116 cells, as confirmed by AUT-PAGE. Therefore, hyperacetylation of histone H4 might not be sufficient for differentiation induction in HCT 116 cells.

4. Both sodium butyrate and TSA caused a time-dependent induction of p21 expression in HCT 116 cells as shown by western blot analysis, which implicates that the induction of p21 is essential but not sufficient for differentiation induction in HCT 116 cells.

5. Sodium butyrate and TSA differ in their effects on RB phosphorylation: upon TSA treatment, RB remains hypo-phosphorylated in HCT 116 (consistent with enhanced expression of p21), but becomes hyper-phosphorylated in the p21 (-/-) derivative. Effects of sodium butyrate-treatment on RB phosphorylation status were weaker in HCT 116 cells. In the p21 (-/-) derivative, where sodium butyrate-treatment still resulted in growth arrest albeit to lesser degree than in wild type HCT 116, hypo-phosphorylation of RB occurred. The mechanisms of the observed differential phosphorylation of RB remain to be elucidated.

6. In addition to p21 expression, we analyzed the expression levels of other cell cycle regulating proteins. Upon butyrate-treatment, expression of the CDK inhibitors p27 and p57 was also increased in a time-dependent manner. The level of underphosphorylated RB slightly increased after 24 h treatment, whereas the expression of E2F1 was significantly reduced. By co-immunoprecipitation analyses using RB antibody, we could demonstrate reduced E2F1/RB interaction. This is most likely due to decrease on E2F1 protein levels, but not a result of modifications in the phosphorylation status of RB.

7. Despite the differences on RB phosphorylation, experiments using DNA array technology (SuperArray@) demonstrated an overall similar mRNA pattern in butyrate- and TSA-treated

samples. Egr-1 and p19 mRNA expression was significantly increased by treatment with both compounds, whereas transcription of cdk6, cyclin D2, p53 and skp1 genes were differentially regulated. Interestingly, similar to the decrease in E2F1 protein levels, E2F1 mRNA levels were also significantly reduced by both compounds as shown in our microarray results.

8. Using HL-60 as differentiation model, we could demonstrate that dihydrohelenalin acetate induces HL-60 cell growth inhibition and differentiation along the monocytic lineage.

9. By the SuperArray@ technique, various key factors for cell cycle regulation were found alternated at the transcription level, which suggest their possible roles in DHAc-induced differentiation of HL-60 cells.

10. DHAc was found to demonstrate a striking suppression of oncogene c-Myc protein time- and dose-dependently in DHAc-treated HL-60 cells, suggesting DHAc as potential therapeutic in cancer therapy.

11. Time- and dose-dependent changes of several cell-cycle key regulators were confirmed at the protein level. While p21 and p27 expression was elevated upon DHAc-treatment after 12 hrs, the multifunctional transcription factor, E2F1 level was decreased after 6 hrs. A significant hypophosphorylation of RB protein was also found after 12 hrs of DHAc treatment. These changes might be a consequence of c-Myc down-regulation.

12. Our result of AUT-PAGE analysis showed that there is no induction of hyperacetylation by DHAc-treatment in HL-60 cells. It suggests that DHAc does not inhibit activity of histone deacetylase.