Reviewer's report

Title: Global Gene Expression Analysis in Time Series Following N-Acetyl L-Cysteine Induced Epithelial Differentiation of Human Normal and Cancer Cells In Vitro

Version: 1 Date: 15 November 2004

Reviewer: Pierfrancesco Marconi

Reviewer's report:

General
In this manuscript Dr. Gustafsson and colleagues examined, using microarray technology, the changes in gene expression accompanying the antiproliferative and differentiating effects induced by NAC in two different cell types, normal human epidermal keratinocytes (NHEK) and the epithelial cancer cell line Caco-2. In the two cell types the genes affected by NAC are quite different and only a very few genes (7) are regulated similarly. The fact that NAC inhibits proliferation and accelerates differentiation of NHEK and Caco-2 cells is interesting and adds further information about the potential role of NAC in tumor prevention. The question posed by the authors is new and well defined and the methods are appropriate. The paper can be accepted after the indicated revisions.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1) The two in vitro proposed differentiation models have been reported in a manuscript that the authors cite as submitted. Since this is not available to the reviewers, a more detailed description is necessary.

2) The authors state that genes affected by NAC are largely related to growth arrest and stimulation of differentiation in both cell types examined. To better understand the biological significance of the changes in gene expression, a table with the classification of genes in the database in functional categories when possible, should be given indicating in each category the percentage of genes that were upregulated/downregulated during the processes examined.

3) The fact that the responses of the two cell types are very different and only 7 genes are regulated similarly could be due in part to the different NAC concentrations used, 2 mM in NHEK and 10 mM in Caco-2 cells. Since gene regulation can be strongly influenced by NAC concentration, why have the authors used two different concentrations?

4) On the basis of the known antiapoptotic activity of NAC and the observations of the authors that the inhibition of proliferation and the accelerated differentiation induced by NAC occur without induction of apoptosis in both cell systems, did the authors examine the expression of Bcl-2 family genes or other genes involved in apoptosis regulation and execution? Even if these genes are not affected by NAC, this should be clarified in the manuscript because some pathways of cell differentiation can lead to apoptosis.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1) In the second paragraph of Background the authors state that a NAC receptor at the cellular membrane has been proposed: by whom, where and when?

2) In References ≠ 2 and 4 the volume and pages are missing.

Discretionary Revisions (which the author can choose to ignore)
None

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**
I declare that I have no competing interests