In this thesis the applicant is focusing on understanding the mechanisms of apoptosis induced by the immunological apoptogen TRAIL in a variety of colon cancer cell lines using different conditions of cultivation. The thesis encompasses a large volume of work and a lot of results. I have a few concerns, though, which are spelled out point-by-point below.

1. In general, the thesis is written in good style, showing a good command of English of the author. The author has written the thesis in a rather mature manner, which makes it easy to read and understand the points of the project, the results and the discussion.

2. The title of the thesis is somewhat vague and should better reflect the major focus of the work.

3. The abstract is good, although its 'introductory' part is a bit too long; in general, more space should be given in the Abstract to the actual results. A final sentence indicating some future perspective or outcomes of the thesis could be included.

4. The abbreviations should be better edited, since some of them should not be included. For example, abbreviations or acronyms like DNA, e.g., His, N-terminus, p53, or σ, should be removed form the list. Also, only expressions used more than 2 times should be abbreviated.

5. The Contents is too detailed in terms of the subdivision of the individual parts. For example, the author need not include parts like II.2.2.2.3.1 as a separate part but it can be within part II.2.2.2.3, etc.

6. The literature review is written well, indicating a good grasp of the topic by the author. The part on the TRAIL receptors should be a bit more detailed, perhaps, since the initially accepted role of TRAIL decoy receptors has been currently somewhat challenged. Since these receptors exert affinity for TRAIL, they ought to be of importance. The part on apoptosis signalling is written well, with sufficient detail, again, indicating a good understanding of the topic by the author. Figure II.2 should be bigger, since it is hard to see its details. Also, use of colour would be of advantage here. Figures II.3 and II.4 are too small as well. The part on cancer stem cells II.2. is written well, given the difficulty of this area of research, which is affected by ongoing discussion what is a cancer stem cell, what its origin is, and, indeed, whether such a cell does exist at all. The two citations in the footnote on page 28 (Neuzil et al., 2007; Clarke, 2004) are not included in the References at the end of the thesis. The chapter on Markers of CSCs (II.2.2.2) would benefit from inclusion of a table with an overview of the markers.

7. The Materials and Methods section is written well and with sufficient detail. Also, the choice of methodology shows that the author has developed expertise in a variety of techniques of cell and molecular biology. One point I would like to make concerns the conditions for spheroid (sphere) cultivation. First, I wonder why the author uses the term 'spheroid' rather than 'sphere'. This point is really minor only. Second, and more importantly, of course: to prepare the sphere phenotype, the author replaced the serum-containing medium with the serum-free medium. This may be a problem, since the cells undergo a shock, which would effect the final result. Our own experience is that a preferred approach is the so-called 'weaning', i.e. a sequential change of the medium (serum containing to serum-free, 3:1, 1:1, 1:3, and, finally 100% serum-free medium). We adopted this approach according to Reynolds and Rietze (2005) (see References),

also following personal discussion with the first of the author. This is a much more gentle way of preparation of spheres and we have good experience with this method. We also noted that the pattern of changes in some selected genes is different when changing the conditions abruptly and gradually. Surprisingly, the Materials and Methods section is not referenced at all, which would indicate that the author developed all methods himself, which surely is not the case. Some parts of this section can be taken out or at least shortened, e.g. part III.1.3, since freezing and thawing of cells is a basic method of cell biology not worth the space. Small typographic problems occur in the section: for example, '300000' should be typed as '300,000', 'hours' should be 'h', '0,5 μg/ml' should be '0.5 μg/ml', etc.

8. The Results section is, again, reasonably well written. I would question the selection of genes the author selected to analyse in the cell lines cultivated in serum-containing and serum-free medium. For example, I would expect to see analysis of the two TRAIL decoy receptors (DcR1/TRAIL3, DcR2/TRAIL4). The inhibitor FLIP should be analysed for as well. All analyses were carried out on the protein level (using flow cytometry). RT-PCR (or better Q-PCR) analyses should be included as well. Some characterisation of spheres, wherever formed, should be included, such as the average diameter, number of cells, viability, etc.

9. The second part of the Results section, i.e. the co-cultivation studies, is interesting, although in my opinion, it would be better to focus more on the first part and analyse the various types of cells (grown as adherent cells and as spheres). As the thesis stands now, it lacks focus to some extent.

10. Throughout the Results section, the figures should be bigger, some of the details are a bit hard to decipher.

11. The Discussion part is OK, given the results obtained. The greatest problem is that the results section does not really give any clear-cut results, showing a rather heterogeneous data for the various conditions tested. This may be due to the, in my opinion, a bit diffuse focus of the thesis.

12. The reason for inclusion of the Epilogue part completely eludes me.

13. The points mentioned above are meant to help improve the thesis and help the author in his further research aspirations. Of course, the author has shown his ability to establish a variety of methods, understanding the topic and the ability to work more-or-les independently, and he should be allowed to defend his Diploma Thesis.

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