Reviewer's report

Title: Metabolic and morphological alterations induced by proteolysis-inducing factor from Walker tumour in C2C12 myotubes.

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Reviewer: Kent KL Lundholm

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General

This study has evaluated possible effects by a proteolysis-inducing factor (PIF) assumed to be produced by Walker tumours in rats. The study is potentially interesting but is poorly focused and the methodology may be questionable in some aspects. Besides, the manuscript does not read well, due to long cumbersome sentences and partly poor English.

The major problem with this study is actually that it is unclear to what extent Walker cells in themselves contribute to production of the so called WF factor. It may well be entirely host produced by a peritoneal based transudate. Therefore, it should have been interesting to include some control experiment performed by any other i.p. irritating and ascites producing factor different from malignancy. The WF factor was evaluated at increasing concentrations by cell viability tests, nucleic acid contents, chymotripsin like activity, protein synthesis, protein degradation and ROS formation. However, time course information is not provided in graphs for any of the measured variables. Therefore, it is difficult to evaluate whether these reactions are time proportional. This is particularly important for measurements of protease activity, protein synthesis and protein degradation. It is also unclear why the WF factor should create increased NAC levels at 24 and 48 hours, and then decreased levels at 72 hours, although the increased levels were not indicated as statistically significant. Anyway, it is difficult to understand why nucleic acid content should increase between 3-15 mg of WF at 24 hours incubation. Does WF stimulate cell replication?

It is also difficult to accurately measure protein synthesis by using L-[2,63] phenylalanine which is an extremely labile isotope usually containing a large amount of the radioactivity in soluble form (water) due to 3H transfer. Thus, unspecific labelling of proteins may occur under these circumstances (incorporation from 3H2O). It is also questionable whether protein degradation can be meaningfully evaluated by muscle proteins prelabelled by tritiated phenylalanine. If so, soluble released material (radioactive) should be characterized in any way. (This assay does probably involve lysosomal enzymes as well).

The purification procedure of the WF-factor is unclearly described. Was the WF-factor precipitated by 40% ammonium sulphate, washed and then re solublized before ultrafiltration?? This seems unlikely. It is also obvious that the factor is biologically active only at considerably high concentrations (approximately 1mmol/l).

Both the Introduction and the Discussion are written with broad perspectives on cancer cachexia. It should be more appropriate to focus on biochemical aspects of identification problems of the WF factor and the methodological aspects. To this review it seems most likely that PIF-activity, originally reported to be produced by the MAC-16 tumour in mice, and now in Walker 256 bearing rats is a host derived protein, which may be just secondary to inflammation in both murines and some cancer patients. This should be an interesting focus of a revised manuscript.

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

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

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Discretionary Revisions (which the author can choose to ignore)
What next?: Reject because scientifically unsound

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interest.

Kent Lundholm