Reviewer's report

Title: Cystatin E/M suppresses legumain activity and invasion of human melanoma

Version: 1 Date: 18 June 2009

Reviewer: Thomas Reinheckel

Reviewer's report:

Cystatin M/E (CST6) is a cysteine protease inhibitor with established binding to the proteases legumain and cathepsin L/L2. Cystatine M/E was originally found in mammary cancer and a functional role of cystatine M/E in cancer has been suggested; however, there are contradictory reports on this. Hence the topic of this article is timely and interesting. Using a panel of metastatic and non metastatic melanoma cell lines and analyzing these for expression/activity of proteases and cystatin ME is certainly a good approach to solve the issues on Cystatin M/E and cancer. The second part of the paper (Fig. 3 and 4) shows overexpression of cystatin M/E in cystatin M/E negative cell lines resulting in reduced legumain activity and reduced invasion.

However, there are some concerns on both parts of the paper that require major revision.

1) Figure 1 shows an association of cystatin M/E expression with legumain activity in cell culture supernatants of a panel of cell lines. However, some cell lines shown in A are missing in the activity measurements in B. Why are these cells omitted? Results need to be shown.

2) The results of Figure 1 are hard to interpret in the context of the results shown in Figure 2. Cell lines MCC13, MCC57, and MCC72 show low legumain activity and high cystatin M/E (Fig. 1) but also very low legumain protein level in cell lysates. Hence, it is not clear if the low legumain activity in the cell culture media is due to inhibition by cystatin M/E or due to low expression and secretion of the protease. To solve this specific legumain activity (legumain activity / legumain protein concentration) needs to be determined in the conditioned media.

3) To strengthen the point of the paper, the protease and cystatine levels should be further correlated with the invasiveness of the cell lines measured by Matrigel assays (as presented in Figure 4).

4) Cathepsin L has been shown to interact with cystatin M/E at a site distinct from legumain. Furthermore extensive work from Frade and colleagues suggests cathepsin L involvement in melanoma. Hence it would be highly reasonable to include cathepsin L activity measurements & western blots in the analyses of Figures 1 and 2; but especially in the more functional investigations presented in Figures 3 and 4.
Nevertheless, I feel this paper could nicely contribute to the literature on proteases/protease inhibitors in cancer – given the above mentioned points being addressed.

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

**Quality of written English:** Acceptable

**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 interests