Reviewer’s report

Title: Protein-bound polysaccharide from Phellinus linteus inhibits tumor growth, invasion, and angiogenesis of SW480 human colon cancer cells by modulating Wnt/beta-catenin signaling

Version: 1 Date: 26 May 2011

Reviewer: Gabriella Calviello

Reviewer’s report:

In this manuscript the authors have observed that polysaccharides extracted from the Phellinus linteus (PL) inhibits the proliferation of SW480 cells in vitro and in vivo, the proliferation of HUVEC in vitro and the tube capillary formation of HUVEC in vitro, the invasion and motility of SW480 cells in vitro, the SW480 cell growth in vivo, as well as the apoptosis and angiogenesis in the tumors obtained from SW480 injected in vivo. They correlate all these effects to the inhibition of -catenin expression and transcriptional activity caused by PL. The PL-induced decrease of the expression of cyclin D1, a downstream -catenin-target gene corroborates their hypothesis.

Minor Essential Revisions

The article is clear and, on the whole, well written. The findings are interesting, but a title like: “Protein-bound polysaccharide from Phellinus linteus inhibits tumor growth, invasion, and angiogenesis and alters Wnt/-catenin signalling in SW480 human colon cancer cells” seems more appropriate.

Background:
Page 3, lines 7-13 : “Cancer invasion and metastasis consist of several interdependent……” This paragraph can be omitted.
Page 3, line 15. Change into: “The polysaccharide isolated from Phellinus linteus (PL)"
Page 4, line 4: Change into: “One important signaling pathway involved in the etiology…”
Page 4, line 16: Change into: “In the present study, we have investigated the effects of a PL treatment on ….”.
Page 4, lines 21-22: Change into: “Our data suggest that the PL-induced down-regulation of Wnt/-catenin signaling may contribute to the inhibition of tumor…."

Results and Discussion:
The concentration-dependence of cyclin D1 expression inhibition (fig 1B) is not so clear, the authors should add the cyclin D1/ -actin ratio value for each blot
The different results obtained with the two different conditions intravenous or the intratumoral injections of PL are not explained neither in the Results or in the Discussion. Moreover, it would be better to specify in Fig 4: “PL intratumoral injection” (in panel A) and “PL intravenous injection” in panel B.

Page 9, last line, page 10 lines 1-4: When the author assess the effect of PL on proliferation by using the MTT assay, do they have also evaluated if PL (especially at the high concentrations) is cytotoxic (i.e. by measuring the percentage of died cells in the cell culture, for instance using the trypan bleu exclusion assay)? They write: “We previously demonstrated that PL has an anti-proliferative effect for SW480 colon cancer cells and the growth inhibition is mediated by induction of apoptosis and G2/M cell cycle arrest”. But it is important also to evaluate whether PL exert cytotoxic effect (by causing necrotic death).

Page 10, lines 9-11 “Depending on the presence of APC mutations and #-catenin over-expression in SW480 cells, [33] the....”: Change into: “We examined the potential effect of PL on #-catenin protein level and activity in SW480 cells, since the se cells are reported to carry mutations of APC and over-express #-catenin [33].”

Discretionary Revisions

The observation that MMP-9 activity is inhibited following the treatment with PL is an interesting finding, but seems not to correlate with the inhibition of #-catenin overexpression caused by these compounds. Thus, it does not add strength to the main hypothesis (the involvement of the #-catenin pathway in the anti-cancer effects of PL). On the other hand, it has been previously reported that the expression and activity of MMP-7 is regulated by #-catenin in human colon cancer cells (Brabletz et al 1999, Am J Path 155: 1033-1038). Thus, it is surprising that the authors did not evaluate the activity of MMP-7 in colon cancer SW480 cells, but, instead, those of MMP-2 and MMP-9.

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.