Reviewer's report

Title: Human desmoid fibroblasts: matrix metalloproteinases, their inhibitors and modulation by Toremifene

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Reviewer: Erik (Rik) Thomspn

Reviewer's report:

General

The authors have built on previous analyses with these two cell lines which showed that Toremifene reduced the levels of TGF-b1. They provide a considerable amount of data here supporting the antifibrotic nature of Toremifene, and clarifying how this is achieved. Their conclusions regarding reduced collagen levels are well based, however, the specific mechanisms at play are not proven. There are other collagenolytic enzymes not assessed. Use of general class-specific protease inhibitors may help confirm the results, etc. The work is otherwise important and relevant, and makes a significant contribution.

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

1. In the description of “Collagenase Activity” methods (top of page 8), the cells were cultured in MEM or MEM containing Toremifene in ethanol. The control should have ethanol, as described in the “Preparation of Conditioned Media” further down the page. Is this an oversight in the text (in which case it should be corrected) or an oversight in experimental design (in which case it should be repeated).

2. There are a number of problems with the zymography components, as follows: (i) It is my understanding that collagen becomes denatured to gelatin when incorporated into the SDS-PAGE gel, and thus the zymogram becomes another gelatin zymogram. To assess native collagen zymography, a collagen gel or film must be layered on top of the SDS-PAGE gel after washing. Thus, Fig 5 and Fig. 6 are the same thing (and indeed do look identical although no size indication is given) and if so, one can be dropped. (ii) The text indicates two bands for the desmoid fibroblasts on the collagen zymogram, however only one major band is visible. Also, depending on whether APMA treatment was of the samples or gels, one would expect the latent form, if present, to be converted to active form and one would also expect the two bands to be present on the gelatin zymogram, however this was not mentioned (iii) MMP-2 and MMP-9 both are positive in CM by Western analysis (Fig. 7), however only one major band is shown in zymography and no size indication is offered – which gelatinase is shown, and why is the other one not seen? (iv) The Methods indicates that “Zymogens were activated with APMA”, whereas the figure legends (Fig. 4,5) indicate that “The same zymograms were activated with APMA”. In each case, it is not clear what happened. Were the samples activated with APMA before running, as is usually the case, and as suggested in Methods, or was the zymogram incubated in APMA. If the latter, it is unlikely that further activation would be seen since SDS is already able to unfold the latent MMP and allow activity. If the former, it is notable that all the MMP shown would appear to be in the active form.

3. The change in MMP-2 seen by Western is not reflected in the zymogram (although we can only assume the zymogram band is MMP-2 rather than 9). Further work is required to prove this increase, e.g titration loading and/or ELISA assay.
4. The conclusion that Toremifene caused reduced collagen production, at least type I, is well supported, however, the conclusion that it also increases MMP activity is not well supported. Fig. 4 shows a modest increase in $\frac{1}{4}, \frac{3}{4}$ fragments (although the data quality is poor). Only a modest change is MMP-2 is seen by Western, and not reflected in the zymogram, and this is balanced by larger changes in both TIMP-1 and TIMP-2. Significantly more work would be required to prove that this is indeed part of the antifibrotic effect of Toremifene. Use of general class-specific protease inhibitors may help confirm the results, etc.

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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)

1. proteolytically is misspelled on page 8, line 7
2. In showing zymogram data, it appears that the photo has been reversed. It states in the Methods that the proteinase activity is observed as cleared regions…, however, the data are shown as a dark band – the transformation should be described.
3. In describing the collagen zymograms, it is stated that they contain 0.2-1 mg/ml collagen.
4. It would be good to show the GAPDH data in Fig. 1 as well as the normalisation. Lane 2 must be overloaded?
5. Size markers are not indicated on many of the panels. These should be added to Figures 1, 2, 5, 6, 7 (A-C), and 9.
6. More of the discussion should be devoted to Toremifene and how it may be regulating TIMPs, etc.
7. RNA levels for MMP-1 are shown, however, no MMP-1 protein is seen. This is not discussed and should be.
8. Densitometry of the collagen fragmentation is provided for Fig. 2 but not for Fig. 3, although it may be missing because my page printed sideways??). This is an important effect of Toremifene for this study, and should also digitised. The quality of this figure is also suboptimal, especially compared to Fig 3. It would be helpful to combine Fig. 3 and 4 (using samples from the same experiment), so as to see whether Toremifene increased the level of degradation further than control.

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Discretionary Revisions (which the author can choose to ignore)

1. Some of the references are somewhat outdated now, e.g ref 27 for MMPs, and some of the other MMP references. The authors may like to update.
2. It would be interesting to test other anti-estrogens in this system, obviously outside the scope of the current study.
3. It would also be interesting to see whether Toremifene will affect normal fibroblasts in the same way, since it was always used on desmoid fibroblasts in the current study.
4. The first section in Results: Oestrogen receptor assay, would appear to be previously published data from the same group, and would usually be placed in Introduction or Discussion, rather than Results?
5. The use of the term “is only apparent” on page 16 is technically correct, but confusing because of the multiple meanings of apparent. It would be better to replace the term with “is not consequential” or similar, to avoid confusion.
6. Also, in the same paragraph, it would be better to state that TIMP-2 inhibits all MMPs but is 10-fold more potent against MMP-2, and it would be good to provide a reference for this – it is not widely known.
What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

None