Reviewer's report

Title: Activation of Calpain-1 in Human Carotid Artery Atherosclerotic Lesions

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Reviewer: mark Slevin

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This manuscript deals with an important process (apoptosis) associated with the development of unstable atherosclerotic plaques prone to rupture. The authors identify the expression of calpain in endarterectomy specimens, perhaps surprisingly finding the highest levels in asymptomatic patients. I have the following comments:

Major compulsory revisions:

1) When describing the selection of the plaque specimens obtained, the authors do not describe the histological features of each of the samples. This is of critical importance since the neointimal tissue is heterogenous throughout and will also vary considerable along the length of the specimen. A clear description of the cellular composition, foci of inflammation and angiogenesis and regions of haemorrhage and calcification/fibrosis is required before determination and further processing of portions of the samples for Western blotting. I would also suggest that multiple segments along the length of the specimens were dissected and compared.

2) TUNEL is a method which has been shown to bind to both apoptotic and necrotic cells. If the authors want to identify cells undergoing apoptosis they should chose a marker specific for this process e.g. caspase-3 or PARP

3) The authors have compared calpain expression in 29 endarterectomy specimens and found its presence in all of them. This is not surprising but their findings that it is higher in asymptomatic patients is. Apoptosis of cells within complicated neointimal lesions usually results in a loss of stability and may increase the likelihood of thrombosis. I think part of the problem is the fact that the samples of tissue chosen for Western blotting may not have been representative of the susceptible areas and may also not match up with the sections stained by IHC. The authors should also have compared these advanced plaques with less advanced lesions of type III-V and identify if calpain levels were higher or lower in these lesions.

4) The immunohistochemistry pictures are low power, It is neccessary to view higher power pictures in order to identify which cells are undergoing apoptosis (or necrosis). Also, double immuno-staining is required before a statement can be made suggesting that TUNEL staining co-localizes with calpain positivity. Some of the TUNEL staining looks like non-specific background on these pictures.
Minor essential revisions:

1) On page 3 please define in full HMEC

2) On page 4, where a reference to the histological characteristics of plaques is made (reference 17). Much more detail should be provided here.

Discretionary revisions:
None

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

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