Reviewer’s report

Title: Frequent expression loss of Inter-alpha-trypsin inhibitor heavy chain (ITIH) genes in multiple human solid tumors: A systematic expression analysis

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Reviewer: M Mitzi Brentani

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The study by Hann et al investigates the differential gene expression of the ITH gene family in several tumors. As an initial approach to the problem, cancer profiling arrays analyses was appropriately used (241 tumor samples). In particular more detailed analysis of ITH2 expression was performed in an additional set of breast cancer samples. They found that ITH genes are generally down-regulated in several tumors. These results suggest that ITH members might play an inhibitory role in cell growth and spreading derived from their stabilizing effects on the ECM. However until now, the only member presenting a clear negative association with prognosis is ITH5.

The addressed question is interesting as the parallel expression of several ITHs in the same tissue may suggest interactions between these protease inhibitors, suggesting that they may participate in specific pathways. Overall this paper contains new findings and the experiments were carefully done.

However, in contrast to their recent publication (Oncogene, 2007) on hypermethylation of ITH5 in nod negative breast cancer and its association with outcome, the present communication is descriptive and lacks information about the possible mechanisms regulating ITH genes expression. Significant correlations between all ITH mRNAs studied in the 36 breast carcinoma samples and in other cancer tissues was not presented. In the tissue array containing 185 invasive breast carcinoma samples, at least a simultaneous analysis of ITHs and ITH2 should be included to establish clearly a correlation between these ITH family members.

ITH2 loss of expression did not affect significantly overall survival (p = 0.385) as does ITH5 (previous results of the same group). However it is possible that the combined expression of ITH2+ ITH5 or other members may have a better applicability.

Both genes showed a close correlation with ER positivity determined by immuno histochemistry suggesting that this group of patients represent a “typical” breast cancer population, but it will be important to understand the functional relationship between these two proteins. Was the promoter of these genes tested for the presence of hormone response elements? Hormonal regulation of the discussed genes was previously determined in cells culture? There was no attempt to determine whether these in vivo findings have any parallel in vitro. It
will be important to characterize if the expression of ITH family members is affected by hormones. In summary there are several positive remarks (a clear change is the expression in tumors of ITH members as compared with non-tumoral cells; a weak trend between loss of ITH2 with prognosis demonstrating that the family members have not the same value for prognosis and a confirmation of a strong association with ER, previously seen with ITH5), but some new informations should be added or discussed before publication could be possible.

This reviewer expects that this descriptive report is the beginning of a series of comprehensive studies on changes in the ITH family gene profile during malignant transformation of breast epithelial cells that may lead to practical applications.

**What next?:** Accept after minor essential 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, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests