Author's response to reviews

Title: Elevated MED28 expression predicts poor outcome in women with breast cancer

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Author's response to reviews: see over
Dear Dr. Alam:

Thank you very much for your review of our manuscript entitled "Elevated MED28 expression predicts poor outcome in women with breast cancer" by Nam K Yoon et al. We appreciate the comments by all three Reviewers. We have addressed all of their points and questions below. In addition, as you and Reviewer 3 requested, we have highlighted the differences between this current study and a previous study (Expression pattern of the novel gene EG-1 in cancer). It should be emphasized that, in contrast to the third reviewer's comments, this current study substantially advances the field and supplies a novel view of the predictive power and potential clinical utility of MED28 (EG1) in breast cancer progression.

If you have any questions or concerns, please don't hesitate to contact me.

Sincerely,

Lee Goodglick

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Reviewer Gloria Calaf

We thank Gloria Calaf for the review of this manuscript. She stated no major criticism of the paper.

Reviewer: Gulisa Turashvili

1. The Reviewer asked why did some patients have more than one case. The answer is that 32 patients had more than one surgical procedure for which the Department of Pathology received tissues.

2. For a given case, on average 3 spots were taken for each identified histopathology. For example, if we had blocks from a woman who had surgery for breast cancer, we would try to get 3 representative spots of the cancer and 3 representative spots of adjacent "normal" glandular epithelium. To save journal / article space, this is described in the listed references; however, we are happy to state this explicitly in the Methods if recommended.

3. The Reviewer wanted additional information regarding 179 cases with invasive cancer (note: on the patient level, 157 patients had invasive carcinoma out of the 210 patients on the TMA who had surgery). Of those 157 patients with invasive carcinoma, we had primary and metastatic tissue on our TMA from 64 patients. Of the remaining patients who had tissue on our TMA, 29 were from breast reduction surgeries, 17 were DCIS, 3 were ductal hyperplasia, and 4 were intraductal papilloma.

4. Survival data was available for 88 of the 157 patients with invasive carcinoma.

5. Antigen retrieval was used as part of the IHC protocol. This is now included in the methods.

6. Her2 was detected using the Dako HER2 antibody that is included in the HercepTest Kit (catalog number K5204). This is now included in the Methods.

7. The Reviewer has some questions about the scoring system that we used and the cellular components scored. As the Reviewer points out, there was both nuclear and cytoplasmic staining. We scored each compartment separately. The staining patterns of both cytoplasmic and nuclear staining were highly correlated. Therefore, so as not to be redundant, we put the results from cytoplasmic staining in the body of the manuscript, and we included the nuclear staining results in the Supplement.
   b. "What was the difference between 1 and 2 staining intensities". Staining at 1 was weak; staining at 2 was moderate. This is now in the Methods.
   c. "Was 0 negative or weakly positive". We define "0" as "below the level of detection. This is a conservative statement showing that it is negative for IHC (but more sensitive techniques e.g., qPCR, may or may not be able to detect some expression.
   d. "By the percentage of glandular cells, do the authors mean tumor cells?" The answer is not necessarily. If we are examining morphologically normal glands, then we are speaking about glandular or ductal epithelium. If we are looking at tumors, we are talking about malignant cells. However, we are not scoring stromal cells, myoepithelial cells, endothelial cells, muscle, nerve, etc. (which, by the way, were negative for expression anyway).

Results

1. As the Reviewer suggests, we have specifically stated in the text that survival analyses was conducted on a maximum of 88 patients (see Methods).
2. (questions about nuclear versus cytoplasmic staining; refer to the answer above). Survival and recurrence analyses based on the integrated staining (frequency and intensity) of cytoplasmic or nuclear staining, were similar (i.e., highly correlated with one another).

3. As the Reviewer suggested, we have added to the legend of Figure 1E, the definition of the numbers (ie., the number of spots in each category).

4. The Reviewer suggested we show a picture of MED28 expression in a metastatic lesion. This is now included in Figure 1.

5. All staining results represent integrated intensity (frequency combined with intensity) (see legend for Figure 1).

6. The Reviewer asks about statistical differences in Figure 1E. The expression level of MED28 in DCIS, IDC, or metastatic lesions, was statistically different from either normal or DH. The only other comparison of significance was DCIS versus metastatic lesions (P=0.040). This later point has now been added to the Figure legend.

7. If I understand the question, we do have survival data for all 66 patients for whom we have recurrence data.

8. We thank the Reviewer for pointing out an apparent discrepancy in Figure 2A. This issue was apparently caused by a PC to MacIntosh incompatibility. Figure 2A has now been fixed.

9. Here we define "early stage" as stage I/II; late stage is III/IV. This is now in the legend to Figure 3.

10. The observation that the Reviewer made that there are few patients with late stage breast cancer in our population (Figure 3) is correct. At UCLA, this is primarily based on the fact that surgical intervention is not warranted in late stage patients; therefore, paraffin blocks are not as abundant.

11. The Reviewer asked how "high" and "low" were defined in Table 1. For Table 1, Figure 2, and Figure 3, high versus low is defined by dichotomization at the 75th percentile. We have now clarified this further in the Methods.

12. We were able to evaluate 152 out of 242 cases for MED28 expression (or lack of expression). The remaining 90 cases had no spots which could be evaluated. The reason for this later situation is that our targeting of a given histology is sometimes off (e.g., we mean to array DCIS, but we miss during the initially coring and, for example, get only adipose tissue). In addition, there are typically limited number of spots fall off the microscope slide as we cut the array.

**Minor Essential Revisions**

1. As recommended, we changed a sentence in the second paragraph of the Abstract to read: "The association and validation of MED28 expression with histopathological subtypes, clinicopathological variables, and disease outcome was assessed"

2. We have changed "death due to disease" to "disease-specific survival".
Reviewer Remi Houlgatte
We thank Dr. Houlgatte for review of our manuscript. The major comment was that our study here does not "improve the knowledge of MED28" further than the study by Zhang, et al., (Clin. Cancer Res., 10:3504, 2004). We admit to being confused by this comment since the two studies are quite different. The paper by Zhang et al., had two goals: 1) to define the specificity of a new anti-EG-1 (MED28) specific antibody, and 2) to examine qualitatively, the general expression of MED28 in a limited number of breast, prostate and colon cancer - benign tissue pairs.

This current study examines a larger patient population using TMA technology linked to a much richer database of pathology, clinical, and outcomes information than that available to Zhang et al. Moreover, in this current study we are able to address the questions of whether MED28 expression levels can supply meaningful information about disease recurrence and outcome.

Therefore, the current study absolutely and significantly adds to the knowledge of MED28 pathobiology and disease association.