Author's response to reviews

Title: Increased staining for phospho-Akt, p65/RELA and cIAP-2 in pre-neoplastic human bronchial biopsies

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Author's response to reviews: see over
We thank the reviewers for their critiques and comments to improve the manuscript. We believe we have taken the necessary steps to address the reviewer’s concerns as detailed below.

Response to Reviewer Concerns:

1). The authors should describe the total number of samples, number of samples in each histological grade.
   A table (Table 1) with this information has been added.

2). The authors include adenocarcinomas in this study, but they do not include any figures for the adenocarcinomas. Addition of the adenocarcinoma figures is required. Also, the authors should describe how they classify preneoplastic lesions of adenocarcinomas.
   A representative adenocarcinoma stained with all three antibodies on serial sections has been added in the new Figure 6. We did not examine preneoplastic lesions of adenocarcinomas from distal lung (e.g. atypical adenomatous hyperplasia) in this study but focused on bronchial IEN that give rise to tumors of the upper airways.

3). The authors should include the clinical and additional pathological characteristics such as age/sex, TNM stage, survival, recurrence and chemotherapy response, and compare protein expression with these characteristics.
   The age and gender information has been added in Table 1. Age and gender characteristics were not significantly different between groups as determined by ANOVA. As the focus of the manuscript is bronchial IEN lesions, the survival, recurrence and chemotherapy response was not included as it is not relevant to these pre-cancerous lesions. The distribution of TNM staging for the carcinomas examined, if known, has been added in the description of samples. There was no clear correlation between tumor stage and staining intensity for any of the proteins examined.

4) On page 5 of the background section, part of the last paragraph may be for the Results section. I recommend to delete the part after “Increased staining for phosphorylated Akt…”
   The suggested change has been made.

Reviewer 2

1). Activation of Akt has multiple consequences leading to the formation of cancer. Because the focus of the ms is on anti-apoptotic pathways, rather than for example invasion, it would be of interest to know the status of phospho-FKHR in the same samples that phospho-Akt was determined.
   We agree with the reviewer that examination of FKHR is of interest. The human bronchial IEN biopsies used in this study have been mostly exhausted, however, making analysis in the same samples impossible and necessitating repeating all stains on a new set of biopsies if this were to be examined. It should be noted that a positive correlation between staining for phospho-Akt and phospho-FKHR has been reported (Balsara et al. 2004 Carcinogenesis 25:2053). This point has been added to the discussion.
2). This manuscript would be greatly strengthened by showing a final figure with the antibodies used in this manuscript on a serial section of at least one sample. The authors appear to be suggesting that there is a link between upregulation of phospho-Akt, nuclear localization of p65/RELA and increase in cIAP2 abundance. This reviewer is going to require that this analysis be done. However, the inability of showing all of the above effects on one serial sectioned sample should not stop the publication of this ms.

Figure 6 has been added containing the requested serial sections of a moderate dysplasia with staining for nuclear p65/RELA and increased staining for phospho-Akt and cIAP2 in the same area of this biopsy. As was noted in the final paragraph of our discussion, the co-localization of nuclear p65/RELA and elevated cIAP2 stain was not a consistent observation. The lack of a consistent correlation between nuclear p65/RELA and increased cIAP2, together with the observation that increased staining of cIAP2 occurs at a later pathological stage could indicate that cIAP2 is not a direct target for this transcription factor in human bronchial IEN. While there was not a consistent correlation between nuclear p65/RELA and elevated cIAP-2 staining, we did observe a correlation between elevated staining for phospho-Akt and cIAP-2. This result has been added to the manuscript.

Minor revisions
Page 10, end of 1st paragraph, change from Figure 3E and 3F to figure 4E and 4F.
This error has been corrected.