IKKε expression as an immunohistochemical marker for diagnosis of high risk flat epithelial atypia (FEA) of the breast

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Introduction

Most breast cancers are carcinomas starting in the epithelial lining of the breast. Ductal carcinoma of the breast is characterized by proliferation of epithelial cells lining the ducts and lobular units of the mammary gland[1]. Flat Epithelial Atypia (a neoplastic alteration of duct-lobular unit in which the luminal epithelium cells are replaced by atypical ductal cells growing in a flat pattern) is an early stage in the development of low-grade ductal carcinoma[2].

Unfortunately, morphology and immunohistochemistry to date cannot distinguish cases of flat epithelial atypia with concomitant breast carcinoma from those without. In addition, distinction between flat epithelial Atypia (FEA) and morphologic mimickers, such as columnar cell change, can be difficult. Therefore, this study aims to identify a immunohistochemical marker that can diagnose FEA. The target chosen is the protein, IKKε. IKKε is an activator of the NF-κB pathway and its expression is associated with cell proliferation and resistance to apoptosis. IKKε has been documented to be over expressed in 30% of the breast cancers[3]. In this study IKKε expression was studied by immunohistochemistry in a cohort of FEA cases with and without carcinoma and evaluated for % of section+ and intensity. Cases of columnar cell hyperplasia (excessive growth of epithelial cells without atypia) were used as controls since no IKKε activity has been reported in hyperplastic tissue.

Hypothesis: We propose that IKKε is consistently overexpressed in FEA of the breast and further distinguishes FEA with with high risk of breast cancer from that without.

Methodology

Biopsy samples from all three pathologies (FEA with carcinoma, FEA without carcinoma and columnar cell hyperplasia) were fixed with formalin and embedded in paraffin. The embedded samples were sectioned with a microtome (4 micrometer thick tissue sections) and subjected to antigen retrieval (exposure of masked epitopes by heat treatment) at pH 6.0. The samples were then immunostained for IKKε in a blind study. An anti rabbit polyclonal antibody was used with 1:200 dilution in antibody dilution buffer (DAKO CD200082). The samples were incubated in primary antibody overnight at 4° C. After washing in Tris-buffered saline with Tween 20, the sections were incubated for one hour with secondary DAKO Envision + peroxidase Rabbit (DAKO K4002). Slides were washed again followed by application of DAB chromogen (DAKO K3467) which reacts with the polymer resulting in a brown colored precipitate where antibody is bound. Hematoxylin was used as a nuclear counterstain. The samples were then analyzed and compared under a Zeiss Axiolmager.M2 microscope for positive staining and correlation with the different pathologies. The images were taken using an AxioCam MRc.

Results

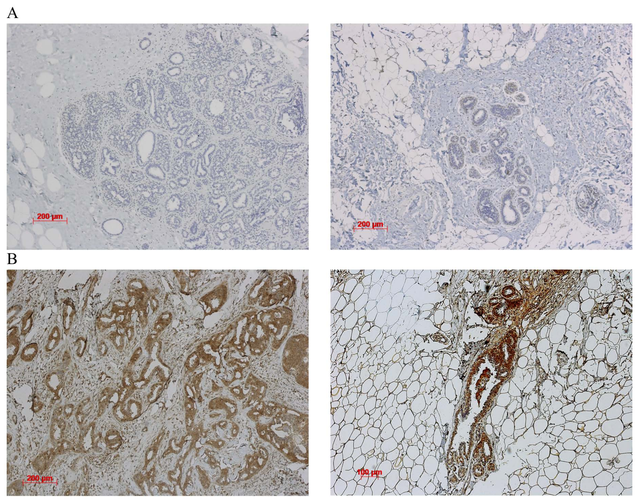

Figure 1. Results of samples after staining with IKKε. (A) Sample with little to no staining indicating the absence of IKKε (5X). (B) Samples with very high staining indicating over expression of IKKε (10X)

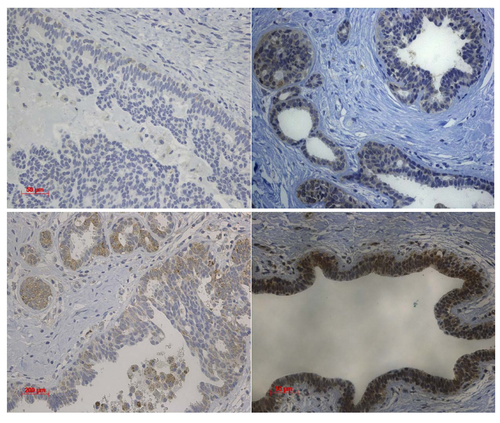

Figure 2. Hyperplastic cells lining the ducts (20X). The expression of IKKε is variable.

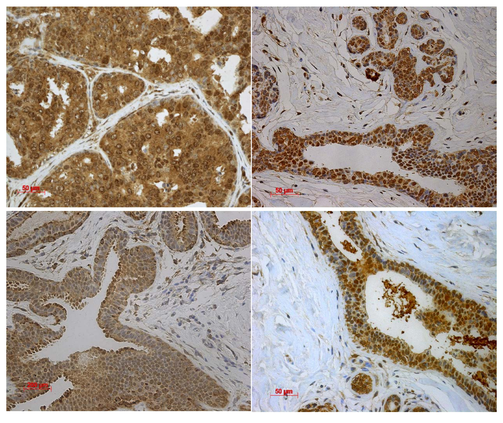

Figure 3. Epithelial cells with flat epithilial hyperplasia (20X). The expression of IKKε is relatively higher.

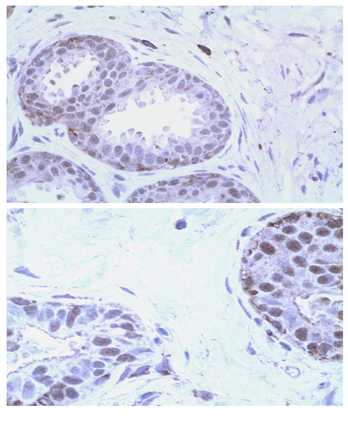

Figure 4. Flat epithelial hyperplastic cells expressing IKKε (20X). The elongated nuclei of cells characterize FEA. (Top) Expression of IKKε in nuclear speckles. (Bottom) IKKε strongly expressed in the nuclei of cells.

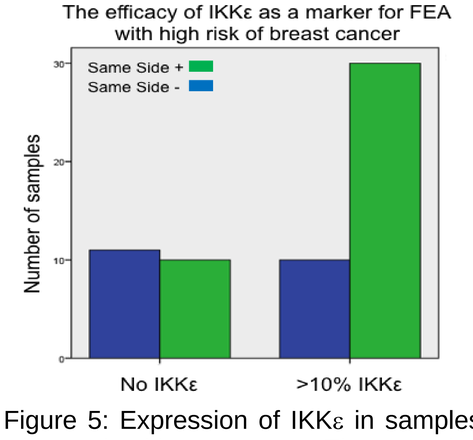

Figure 5: Expression of IKKε in samples with and without cancer. The samples taken from the side of breast with cancer are represented in green. The samples taken from the side of breast without cancer are represented in blue.

Conclusion

After the code was broken the samples were analyzed to discern the expression of IKKε in samples with cancer vs. samples without cancer. The results suggest that the samples taken from the side of breast with cancer demonstrate a high correlation with expression of IKKε. The samples with cancer that do not show high levels of IKKε may not have had FEA as a precursor. The samples without cancer that show high levels of IKKε may have two consequences associated with them. They may be in the early stage of development leading to cancer or they may not lead to the development of cancer. However, since there is a significant correlation between expression of IKKε and FEA associated with cancer, we can infer that samples with high expression of IKKε without cancer have a high risk of progressing to cancer.

The samples for the negative control were not observed so far. Normal mammary gland tissue samples are currently being immunostained to serve as negative control.

From this study we can conclude that IKKε can serve as an immunohistochemical marker for diagnosis of high risk flat epithelial atypia.

References

1. Leonard, G. D., & Swain, S. M. (2004). Ductal carcinoma in situ, complexities and challenges. Journal of the National Cancer Institute, 96(12), 906-920.

2. Lerwill, M. F. (2008). Flat epithelial atypia of the breast. Archives of Pathology \& Laboratory Medicine, 132(4), 615-621.

1. Zhou, A. Y. (2012). Elucidating the regulation and effectors of the breast cancer oncogene, IKKepsilon (Doctoral disseration, HARVARD UNIVERSITY) from http://nrs.harvard.edu/urn-3:HUL.InstRepos:10086028

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