OVEREXPRESSION OF ANTIAPOPTOTIC GENE ASSOCIATED WITH BARRETT'S ESOPHAGUS AND ADENOCARCINOMA OF THE ESOPHAGUS

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INTRODUCTION

- The incidence of obesity and esophageal adenocarcinoma has been increasing in the past three decades.
- Obesity is considered one of the risk factors in the development of EAC.
- Obesity associated metabolic changes results in an increase in insulin-like growth factor-1 (IGF-1), free fatty acid (FFA), and diacylglycerol (DAG) levels.
- FFA and DAG are potent activators of protein kinase C δ (PKC-δ), a novel PKC isozyme ubiquitously expressed among cells.
- IGF-1 enhances the enzymatic activity of PKC-δ. PKC- δ has been characterized as a pro-apoptotic and anti-proliferative kinase as well as pro-survival factor for cancer cells.

Hypothesis and Objectives:- We hypothesize that obesity associated PKCδ overexpression induces anti-apoptotic factors and decreased proapoptotic factors in Esophageal Adenocarcinoma.

RESULTS

- There was significant increased expression of PKCδ, anti-apoptotic factors (Bcl-2 and Bcl-XL) and decreased expression of pro-apoptotic factors (Bad, Bax, and Bak) in BE and EAC samples compared to normal tissues.
- The fold change in mRNA expression of proapoptotic markers Bad and Bak was decreased from 0.94 and 0.91 in Normal to 0.50 and 0.14 in BE and 0.55 and 0.05 in EAC tissues.
- Similarly, the folds change in mRNA increased expression of antiapoptotic factors Bcl-2 and Bcl-xL from 0.99 and 1.19 in Normal to 1.66 and 5.22 in BE and 2.76 and 4.62 in EAC.

MATERIAL AND METHODS

Patient selection:-

- Patients were enrolled at surgery clinics at CUMC-Bergan Mercy Medical Center and CHI Health Immanuel Medical Center.
- Each patient underwent endoscopy with biopsies of tumor.
- We recruited patients with either Barrett's disease or esophageal adenocarcinoma with or without Barrett's disease after IRB approval.
- We collected 19 normal, 8 Barrett's, and 15 EAC tissue samples during endoscopy or esophagectomy.

Sample Processing:-

- Fresh tissue samples were evaluated by a board-certified pathologist for the presence or absence of BE and EAC.
- All esophageal tissue samples were soaked in formalin, dehydrated in ethanol baths, and stored in paraffin blocks. 5µM thick sections were cut from the paraffin blocks for analysis.
- The samples were analyzed for the presence of PKCδ, pro-apoptotic (Bad, Bax, Bak), and anti-apoptotic (Bcl-2, Bcl-XL) factors expression levels by immunofluorescence and RT-PCR. We compared levels of expression between normal, BE, and EAC tissue.

Immunofluorescence:-

IF via deparaffinization, rehydration, and antigen retrieval in Dako Target Retrieval solution. Bad, Bak, Bax, Bcl-2, Bcl- XL, and PKC-immunofluorescence staining was performed with primary and secondary antibodies. Nuclear immunofluorescence staining was achieved with 4′,6-diamidino-2-phenylindole (DAPI). :

Imaging and data analysis:-

An Olympus DP71 microscope was used to scans of the slides and obtain images. The images were measured for immunofluorescence intensity with Image J software (Olympus, St Louis, MO). Statistical significance of the data was calculated by using one-tailed t-tests.

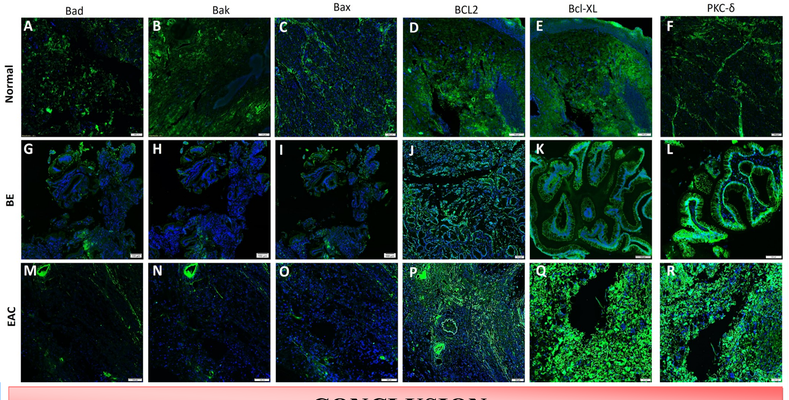
RNA extraction & RT-PCR:-

Total mRNA was isolated using TRI reagent and cDNA was prepared with iScript and GAPDH as housekeeping gene. Real time PCR was performed in triplicate using SYBR green. Fold expression relative to Normal were determined after normalizing to GAPDH expression.

HEMATOXYLIN AND EOSIN STAINING



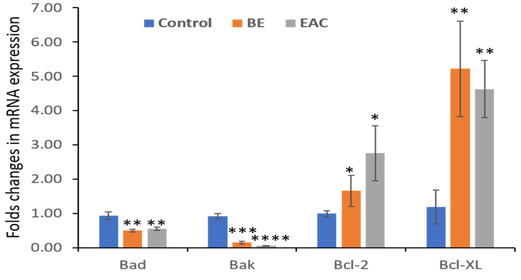
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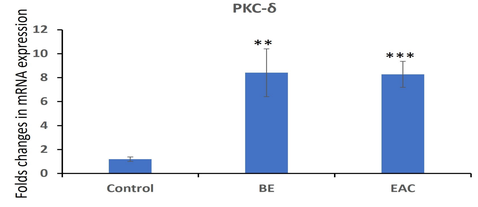


CONCLUSION

An increased expression of PKCδ, anti-apoptotic genes, and decreased expression of pro-apoptotic genes in BE and EAC suggest the role of dysregulated apoptosis in BE and EAC. However, these results need to be further validated with increased number of patients and in-vitro studies.

RT-PCR FOR APOPTOTIC GENES AND PKC-DELTA





PATIENTS DEMOGRAPHICS

Patient Demographics	
Age [mean (range)]	63.56 years old (47-80)
Sex (%)	81.25% (13) male, 18.75% (3) female
BMI [mean(range)]	28.68 kg/m^2 (19.76- 40.5)
Medical History	
EAC staging	No EAC- 18.75% (3) Stage II- 18.75% (3) Stage III- 56.25% (9) Stage IV- 6.25% (1)
Current or prior BE (%)	50% (8)
Current Proton Pump Inhibitor use (%)	100% (16)
Social History	
Tobacco use (%)	93.75% (15)
Alcohol use (%)	25% (4)