Title

Tu1165: THE IMMUNE CELL MICROENVIRONMENT IS DIFFERENTIALLY-ALTERED ACCORDING TO DIVERSE EPITHELIAL LANDSCAPES IN THE PROGRESSION TO CANCER IN BARRETT OESOPHAGUS

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Authors

Lin, Meng-Lay
Hickey, John W
Schürch, Christian M
et al.

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ISOLEVUGLANDINS AND PROTEIN AGGREGATION: A NEW TARGET OF ESOPHAGEAL ADENOCARCINOMA

Ravindran Caspa Gokulan, Kodisundaram Paulrasu, Wael El-Rifai, Jianwen Que, Olivier Boutaud, Sergey Dikalov, Alexander Zaika

Background: The major risk factor for esophageal adenocarcinoma (EAC) is gastroesophageal reflux disease (GERD), which affects a large portion of the adult population in many Western countries. Multiple studies have found that gastroesophageal reflux (GER) causes persistent production of reactive oxygen species (ROS) and other reactive chemicals, which can lead to tumorigenic changes. These alterations occur in normal esophageal cells and precede the appearance of cancerous lesions. Currently, it is unclear how chronic GER contribute to tumor development. For the first time, we have investigated protein aggregation, the hallmarks of neurodegenerative disorders such as Alzheimer's disease, in the progression of EAC. **Methods and Results:** In this study, the exposure of non-transformed esophageal cells with acidic bile salts, that mimics the physiological condition of GERD patients, was found to induce ROS mediated production of isolevuglandins (isoLGs) from lipid peroxidation. IsoLGs are highly reactive with free amines on lysine residues forming LG-lysine lactam protein adducts. This is the first report to reveal the formation of protein aggregates by isoLGs in conditions of GER using multiple approaches including proteostat aggregation assay, amyloid formation, lipid peroxidation assay (C11-BODIPY) and western blotting. A similar induction of isoLG mediated protein aggregation was demonstrated in our two mice models, a surgical mice with esophagojejunostomy, which recapitulates GERD conditions in animals and L2-IL1β mice expressing human interleukin 1β, resulting in inflammation with progression to metaplasia and dysplasia. Analyzing specific biological consequence of protein aggregation, we discovered that the massive accumulation of protein aggregates induces ferroptosis, a type of programmed cell death characterized by the accumulation of lipid peroxides. The specific markers, GPX4 and TFR1, was used to detect ferroptosis in our experimental models. The induction of ferroptosis by protein aggregation was further confirmed using inhibitors, β-cyclodextrin and ferrostatin-1. This is the first study to show protein aggregation and ferroptosis in the early stages of human EAC. We also tested isoketal scavengers and found that these compounds are able to prevent protein aggregation and ferroptosis in human cell lines and mice models. **Conclusions:** This study revealed, for the first time, that gastroesophageal reflux leads to the formation of isoLGs and accumulation of protein aggregates that causes ferroptosis mediated tissue injury. We also found that specific isoLG scavengers efficiently suppresses protein aggregation and ferroptosis in the esophagus, inhibiting oncogenic transformations. IsoLG scavengers may be used to prevent esophageal cancers as a novel therapeutic approach.

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AURORA KINASE A PROMOTES CANCER CELL SURVIVAL BY ACTIVATING UNFOLDED PROTEIN RESPONSE UNDER ONCOGENIC STRESS IN ESOPHAGEAL ADENOCARCINOMA

Heng Lu, Ahmed R. Gomaa, Lihong Bishop, Longlong Cao, Wael El-Rifai, Dunfa Peng, Zheng Chen

Background: Chronic gastroesophageal reflux disease (GERD) is the main risk factor for the development of Barrett's esophagus and its progression to esophageal adenocarcinoma (EAC). EAC is the most common type of esophageal cancer in the United States. Unfortunately, approximately 80% of patients are diagnosed at late stages with a five-year survival rate of less than 5%. We and others have shown evidence supporting non-mitotic functions of AURKA in cancer. Activation of oncogenic signaling leads to a sustained activation of endoplasmic reticulum (ER) stress. Unfolded protein response (UPR) is a pro-survival process, protecting malignant cells from ER stress-induced apoptosis. In this study, we investigated the role of AURKA in promoting cancer cell survival through activating UPR in EAC. **Methods and Results:** Using tumor tissue microarrays, we detected significant AURKA overexpression in EAC compared with normal. Analysis from GEO and TCGA databases demonstrated a highly activated UPR gene signature in EAC samples with AURKA high expression levels. At the meantime, TCGA data analysis and qRT-PCR data of our esophageal tissue samples demonstrated a strong positive correlation of AURKA and BIP, an ER stress marker, mRNA expression level in EAC tissue samples. Using in vitro EAC models of AURKA overexpression and knockdown, we found that AURKA played an important role in activating pro-survival UPR. Western blot analysis demonstrated that AURKA transient overexpression greatly increased, while AURKA transient knockdown dramatically decreased IRE1α protein phosphorylation, expression, and activation, which indicated the activation of pro-survival UPR, in FLO-1 and OE33 cells. Additionally, Western blot analysis indicated that acid bile salts, the mimic of reflux disease in patients with EAC, induced pro-survival IRE1α phosphorylation that was abrogated by AURKA siRNA knockdown. Short-term ATP-Glo and long-term clone formation assay data demonstrated the synergistic effect of AURKA inhibition and Tunicamycin treatment. Western blot and Immunofluorescence (IF) staining data indicated that AURKA protein-bound and co-localized with IRE1α protein in FLO-1 and OE33 xenograft tumor samples as well as in ABS induced BE/EAC mouse models *in vivo*. **Conclusion:** Our data showed, for the first time, that AURKA activates UPR, promoting cancer cell survival during ER stress in EAC. We have shown that knockdown or inhibition of AURKA can significantly reverse pro-survival UPR signaling mechanisms and decrease cancer cell survival. We have initiated pre-clinical studies to test combinations of AURKA inhibitors along with UPR inhibitors. This novel therapeutic strategy could be a promising approach for the treatment of EAC patients.

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THE IMMUNE CELL MICROENVIRONMENT IS DIFFERENTIALLY-ALTERED ACCORDING TO DIVERSE EPITHELIAL LANDSCAPES IN THE PROGRESSION TO CANCER IN BARRETT OESOPHAGUS

Meng-Lay Lin, John W. Hickey, Christian M. Schürch, Emanuela Carlotti, Adam M. Passman, Richard J. Hackett, Shruthi Devkumar, James A. Evans, Philippe D. Gascard, Thea D. Tlsty, Lorenzo E. Ferri, Garry P. Nolan, Stuart A. McDonald

Barrett esophagus (BE) is the major premalignant condition of esophageal adenocarcinoma (EAC). It is characterized by a broad range of distinct glandular epithelial phenotypes determined by gastric and intestinal epithelial cell lineages inside the gland. Glandular phenotype diversity is associated with progression to cancer and is thought to reflect natural selection by the local microenvironment, representing an evolutionary process. However, little is known about cellular populations within the microenvironment, their relationships with the diverse Barrett's epithelium and its alteration in patients who progress to EAC. We characterized the many glandular epithelial phenotypes of BE at the same time as stromal and immune cell types using a highly multiplexed cytometric imaging approach termed co-detection by indexing (CODEX). We performed CODEX using a panel of 56 antibody markers on Tissue Microarrays (TMAs) from 26 BE patients (70 cores of non-dysplastic BE) who have never progressed to EAC (non-progressors) and 6 BE patients (48 cores of non-dysplastic BE from EAC resection specimens) who have progressed to EAC (progressors). Performing cell segmentation and unsupervised clustering on the single-cell dataset, we discovered nine different types of immune cells and 19 distinct types of epithelial cell types. Our data show foveolar cell-only (cardiac) and goblet cell-containing (specialized) Barrett glands are the dominant phenotypes in both progressors and non-progressors datasets. The relative fractions of epithelial cell types per TMA core estimated in both progressors and non-progressors are not statistically different, suggesting the cellular richness of the two datasets was similar. By contrast, progressors had increased numbers of $CD4^+$ Treg cells, $CD8^+$T cells, and M1 macrophages associated with specific epithelial phenotypes—particularly in TMA cores containing a mixture of cardiac and specialized gland phenotypes. Similarly, plasma cells were decreased in the gastric-like and mixed phenotype cores of progressors compared with non-progressors. Interestingly, immune cells are uniformly present in non-progressor cores, irrespective of gland phenotypes; the exception being $CD8^+$ T cells which are enriched in samples with only gastric-type gland phenotypes. We showed for the first time, that a unique immune cell signature ($CD4^+$ Treg, $CD8^+$ T cells, M1 macrophages, and loss of plasma cells) is spatially associated with distinct and diverse epithelial phenotypes in the progression to cancer in Barrett esophagus. These observations may provide cellular biomarkers in developing models of cancer risk in BE patients and understanding the biology of evolution to EAC.

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IN BARRETT'S EPITHELIAL CELLS, APE1/REF-1 IS REQUIRED FOR ACIDIC BILE SALT-INDUCED VEGF EXPRESSION: A POTENTIAL MECHANISM FOR DEVELOPMENT OF SUB-SQUAMOUS INTESTINAL METAPLASIA IN BARRETT'S ESOPHAGUS

Gabriella G. Alvarez, Qiuyang Zhang, Mark R. Kelley, Xi Zhang, Stuart J. Spechler, Rhonda F. Souza

Introduction: Epithelial-mesenchymal transition (EMT) is the process whereby epithelial cells acquire mesenchymal cell features, including the ability to migrate. In earlier studies, we found that acidic bile salts (A&B) triggered EMT in Barrett's epithelial cells via increased production of vascular endothelial growth factor (VEGF), and we proposed that GERD-induced EMT might cause Barrett's cells to migrate under esophageal squamous epithelium to form sub-squamous intestinal metaplasia (SSIM), a potential source of cancers missed by endoscopic surveillance and of metaplasia recurrences after endoscopic eradication therapy. We showed that Barrett's cells exposed to A&B increase intracellular ROS production, resulting in increased nuclear levels of hypoxia inducible factor (HIF)-1α, a transcription factor that induces VEGF mRNA expression. Full transcriptional activity of HIF-1α requires conditions of low reduction/oxidation (redox) potential, and APE1/Ref-1 (hereafter called simply APE1) is a multifunctional protein with redox function that acts to maintain HIF-1α in its active, reduced state. In this study, we explored whether APE1 is required for the A&B-induced VEGF expression that triggers EMT. **Methods:** Barrett's epithelial cell lines (BAR-T and BAR-10T) were treated with a 15-minute exposure to A&B medium (pH 5.5), and we assessed: 1) nuclear localization of APE1 and HIF-1α by immunofluorescence, 2) HIF transcriptional activity by HRE reporter, and 3) VEGF mRNA levels by qPCR. We used siRNA to knockdown APE1, which we confirmed by assessing APE1 mRNA and protein levels. We also used constructs that overexpressed 1) wild-type APE1, 2) a redox-dead APE1 mutant lacking redox function, or 3) a nuclear localization signal (NLS) APE1 mutant that is unable to translocate to the nucleus; these constructs contained an HA tag, which was used to confirm protein overexpression by Western blot. **Results:** Exposure to A&B resulted in co-localization of APE1 and HIF-1α in the nucleus in both BAR-T and BAR-10T cells. A&B significantly increased HRE reporter activity, which was blocked by APE1 knockdown with siRNA and by abolishing APE1 redox function with the redox-dead mutant (Figure 1). In control BAR-T cells and in BAR-T cells overexpressing wild type APE1, A&B significantly increased VEGF mRNA expression; this increase was blocked by preventing translocation of APE1 into the nucleus with the NLS mutant (Figure 2). **Conclusion:** In Barrett's epithelial cells, increased HIF transcriptional activity induced by A&B exposure depends on the redox function of APE1, and nuclear translocation of APE1 is essential for A&B-induced increases in VEGF mRNA. These findings suggest that APE1 mediates the GERD-induced transcription of HIF-1α that results in VEGF production that triggers EMT. Thus, APE1 redox function might be a target for preventing SSIM in Barrett's esophagus.