Chromosomal Translocation Marks 'Point of no-Return' in Barrett's Epithelium Carcinogenesis cell culture model

Manisha Bajpai, Ph.D. [1], Hana Aviv, Ph.D. [2], and Kiron M. Das, M.D., Ph.D.[1]
Department of Medicine-Gastroenterology and Hepatology[1], Department of Pathology[2], Cytogenetics Laboratory,
Rutgers-Robert Wood Johnson Medical School and Cancer Institute of New Jersey, New Brunswick, New Jersey, USA

Introduction

Barrett's epithelium (BE) is a sequel of inflammation resulting from chronic gastroesophageal reflux and is a major risk factor for esophageal adenocarcinoma (EA). Molecular events predisposing to BE pathogenesis are still unclear and stratification of patients at higher-risk for EA remains a clinical challenge. To gain insight into the molecular processes in BE carcinogenesis, we developed a novel in-vitro BE carcinogenesis (BEC) model (Int J Cancer 2011). Benign, hTERT immortalized BE cells (BAR-T) were exposed to acid and bile at pH4 (B4) 5 min each day for 60 weeks (W). Progressive neoplastic changes accumulated e.g., amplification of colonic / intestinal phenotype mAbDas-1$^+$ and CK8$^+$ cells (Lab Invest. 2008), chromosomal aberrations (BEC20W) (Mol. Cytogenet. 2012), change in cell shape with clumping (BEC40W) soft agar colony formation (BEC60W) and finally, tumors in nude mice confirming malignant transformation of cells (Int J Cancer, 2011). A parallel set of BAR-T cells not exposed to B4 did not show these changes.

Aim

Identification of marker(s) of 'point of no return' in Barrett's carcinogenesis in the BEC model

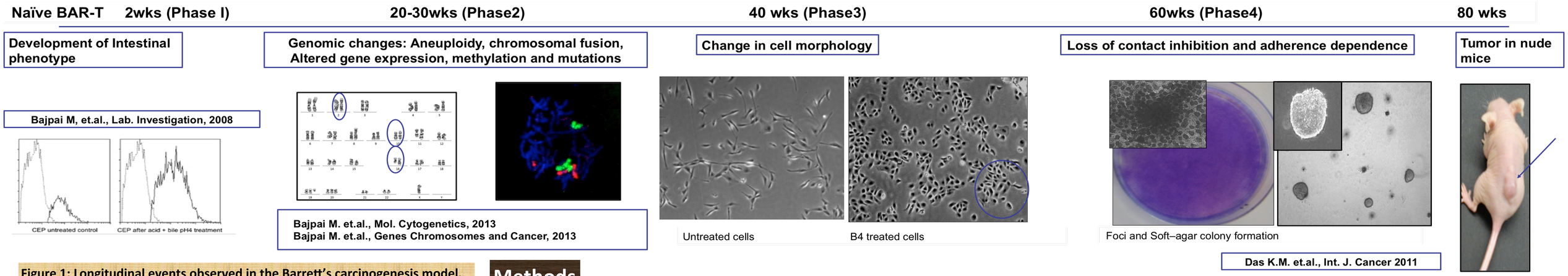

Figure 1: Longitudinal events observed in the Barrett's carcinogenesis model.

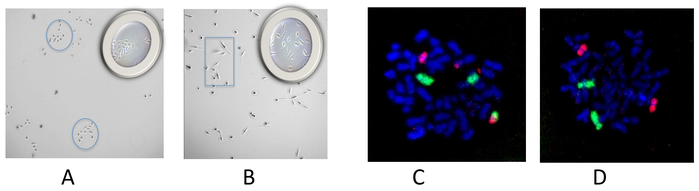

Figure 2: BEC 20W cells in the presence of B4 stimulation for additional 14W develop morphological changes (A)(oval and cohesive / clumped marked by circles from elongated and evenly distributed, marked by rectangle). Chromosomes painting revealed translocation t(2,10,16) appeared around the same time (C). These changes in morphology and chromosomes of BEC20W were not observed in absence of further B4 exposure (B and D).

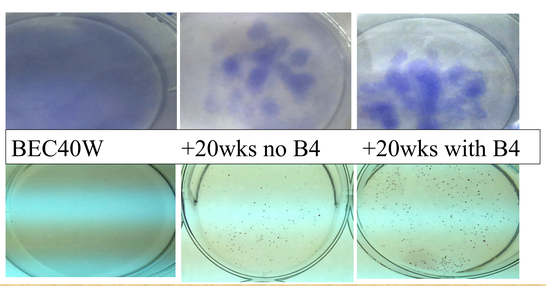

Figure 3: BEC40W grown in the absence of continued stimulation with B4 for 20wks, retain the ability to form cohesive foci (upper panel) and develop the ability to form soft agar colonies i.e., loss of adherence (lower panel) just like the BEC60W cells (BEC40W cells grown for another 20W in the presence of continued B4 exposure).

Methods

BEC20W and 40W cells were revived from storage and divided into two groups, one group was exposed to B4, 5 mins everyday and compared to the untreated group, grown in parallel. After 20wks, cells were assessed for following endpoints : cytogenetic evaluation of chromosomal changes by whole chromosome painting (Molecular probes, USA), change in cell shape, cohesion or clumping of cells and colony formation in soft agar (Cell transformation detection assay, Chemicon, Germany).

Results and Discussions

❖ **Progressive neoplastic changes may be impeded after removal of acid and bile stimulus during early stages of BEC model**

A window period between BEC20W and BEC40W is marked by change in cell morphology, cohesive aggregation of cells and appearance of characteristic translocation event that involves breakage and fusion of non-homologous chromosomes (2,10 and 16). BEC20W cells exposed to B4 for additional 14 weeks (i.e. BEC34W) change to oval shape with characteristic clumping, however those grown without further B4 stimulation retain their elongated morphology and spread evenly on the surface of culture dishes (Figure 2A and B). Cytogenetic evaluation of the cells growing with and without further B4 exposure for the same duration reveals appearance of translocation only in the cells exposed to B4 continuously (Fig 2C and D).

❖ **Neoplastic changes persist even after removal of acid and bile stimulus during later stages of BEC model**

The BEC40W cells retain the oval shape and translocation t(2,10,16) even in the absence of further exposure to B4. They also acquire the ability to form foci at high cell concentrations (inhibition of contact inhibition) and colonies in soft agar at the end of the 20 weeks observation irrespective of further exposure to B4. When BEC60W cells were grown for another 20wks without B4 exposure, they retained the characteristics of malignant transformation.

Conclusions

The BEC model demonstrates that absence of B4 trigger could prevent progression of neoplastic changes in BEC20W cells but not in BEC40W cells that appear to be committed to progressive transformation i.e., **"point of no return"**. This study identified a novel translocation t(2,10,16) [involving chromosome segments harboring known carcinogenic genes] that reliably appears in the BEC34W cells six weeks before the 'point of no return' (BEC40W). Translocations are considered biomarker events in leukemia, prostate and lung cancers, but have not been investigated in BE / EAC. Further investigations will reveal the significance of this chromosomal event(s) in commitment of the cells toward malignant transformation in the BEC model. The t(2,10,16) after clinical validation may be useful as a fluorescent in-situ hybridization (FISH) based predictive marker to stratify BE patients at higher risk of developing EAC.