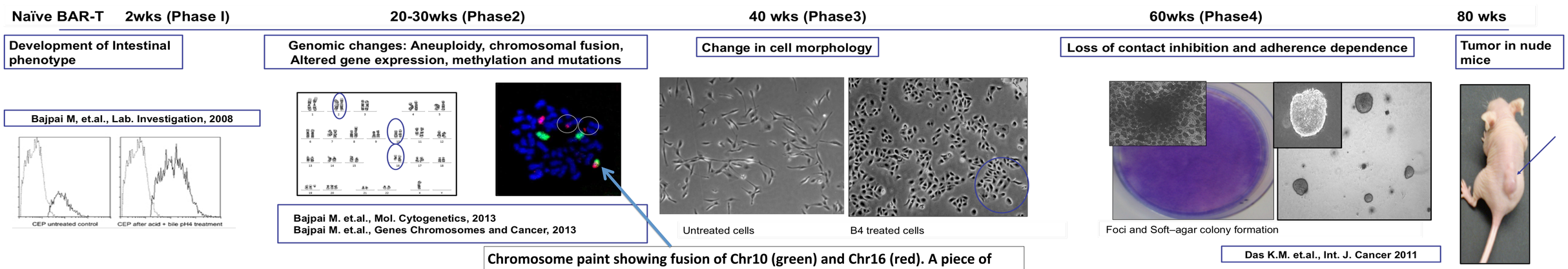
Novel Recurring Fusions: Predictive Markers of Esophageal Adenocarcinoma

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

RUTGERS Robert Wood Johnson Medical School
Biomedical and Health Sciences
RUTGERS Cancer Institute of New Jersey

Introduction

Barrett's esophagus (BE) is a pre-malignant condition at the distal esophagus due to chronic gastroesophageal reflux mediated injury that can progress to dysplasia and Esophageal adenocarcinoma (EA). Patients with BE are at a 120 fold higher risk of developing EA in a slow progression of disease from BE to low grade dysplasia, from low grade dysplasia to high grade dysplasia, and ultimately from high grade dysplasia into EA over several years. Slow progression of the disease allows time for medical intervention to prevent or slow down cancer development. However, since BE is asymptomatic, EA is diagnosed at advanced stages and 5yr survival is less than 15%. Therefore, diagnostic markers predictive of premalignant stages of EA arising from BE are an urgent clinical need. Chromosomal fusion events are considered undisputed markers of disease as they may lead to clinically significant fusion genes, e.g. BCR-ABL, leukemia,TMPRSS2-ERG, prostate cancer and EML4-ELK, lung cancer or, alter the position of a novel promoter, thus facilitating carcinogenesis. We first reported, novel chromosomal fusion t(10:16) in an in-vitro Barrett's epithelial carcinogenesis (BEC) model (Cytogenet. 2012). The BEC model demonstrates aneuploidy, change in cell morphology, loss of contact inhibition and tumor formation in nude mice suggesting malignant transformation following acidic (pH4) bile (Glycochenodeoxycholic acid) or B4, exposure to benign Barrett's epithelial cell line, BAR-T, 5 mins everyday for 60 weeks (BEC60W) (IJC. 2011). Three fusion events: t(2:16); t(2:10); and t(10;16) (Fig 1A) were identified in the BEC model as early as 30 weeks of B4 exposure (BEC30W). These fusions are retained in the malignantly transformed BEC60W cells. These fusion segments harbor known oncogenes and have the potential to contribute to EAC development. Since these events occur earlier in the carcinogenic process, they may be valuable as predictive events in BE progression to EA.



Chromosome paint showing fusion of Chr10 (green) and Chr16 (red). A piece of Chr16 (red) translocated and fused with Chr2 is also seen in the circled area.

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

Hypothesis

If the fusions are early indicators of malignant transformation as observed in the BEC model, they must be present in human EA tumor

Methods

DNA probes spanning the break points of the 3 fusions were identified after screening the human genome bacterial artificial chromosome library (BAC) U.S. Provisional Application Serial No. 62/305,194. Using standard fluorescence –in-situ hybridization (FISH) assay, the BEC0W (without fusions) and BEC30W (with fusions) were used to confirm the specificity of the probes. Paraffin embedded 5 micron EAC tissue sections were then hybridized with these custom FISH probes. Human EA tumor and adjacent normal issues were obtained from the Biospecimen repository at the Rutgers Cancer Institute of NJ.

Results

The customized break apart FISH probes are able to clearly detect all three fusions in the BEC30W cells with 100% specificity and sensitivity (Fig:1b and c). The t(2;16) fusion (Fig 2) was detected in 6 out of 8 human EAC tumors tested so far. Investigation with probes for t(2;10) and t(10;16) is still ongoing.

Discussion

Since the fusions appeared in BEC30W cells that preceded malignant transformation seen in BEC60W cells, they may be early predictor of progression to EA. We have evidence of a recurring fusion t(2;16) in human EA. The fusions t(2:16); t(2:10); and t(10;16) identified in the BEC model after further clinical validation may be useful as "predictive" markers for diagnosis of BE progressing to dysplasia /EA and targets for EA therapy

Study partially supported by GI Divisional funds, Rutgers Research Council grant to MB, IMPACT grant to MB from The Rutgers Cancer Institute of NJ (P30CA072720) and the Award of Hope Gala and/or Jattrude Frogarty Trust.

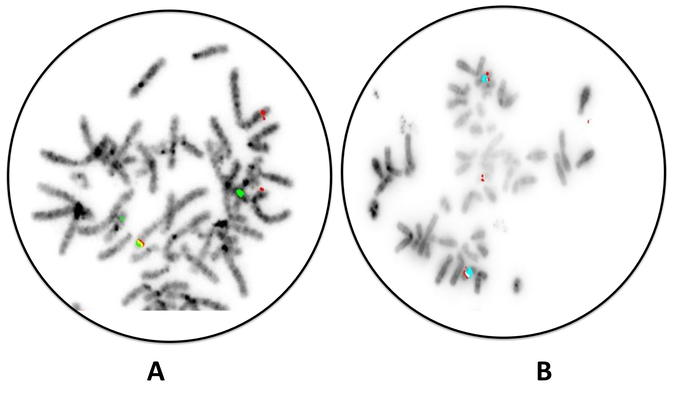


Figure 2: BAC clones can be used as specific FISH probes for the translocation event(s) seen in BEC model. The breakpoint on Chr2p22 and Chr16q22 are spanned by BAC clones (red and green respectively). Normally, the paired chromosome is represented by two red/green dots. Exchange of segments between heterologous chromosomes results in new fusions as witnessed in **Fig:2A** (fusion is yellow). The breakpoint on Chr10q22 is spanned by orange probe, the aqua probe is a label for the Chr10. **Fig:2B** shows the breakage of the orange segment of Chr10 and its displacement to Chr16 (colorless), while the aqua part of the Chr10 remains intact.

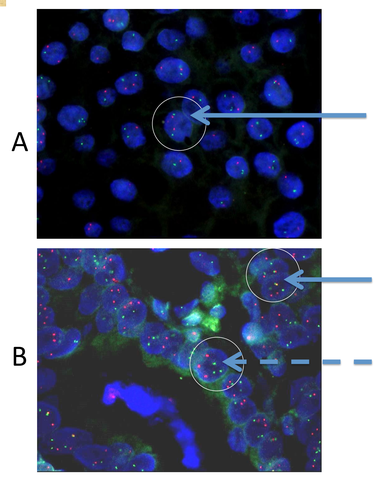


Figure 3: The indigenous FISH probes detect the translocation event in clinical EA tissues. A. The probes spanning the breakpoint on Chr2 (red) and Chr16 (green) (two dots for each pair of normal chromosomes marked by arrow) and **B.** the break apart segments of Chr2 and Chr16 appear as the third red and green dot (dotted arrow) or a fusion resulting in yellow color (arrow).