Genomic Changes Correlate with Transformed Phenotype in a Dynamic *in-vitro* Model of Barrett's Carcinogenesis

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INTRODUCTION

Barrett's epithelium (BE) is a sequel of inflammation at the gastroesophageal (GE) junction due to chronic GE reflux that causes metaplasia of normal squamous epithelium to columnar type. BE is a major risk factor for esophageal adenocarcinoma (EAC). EAC has the highest rate of rise amongst all cancer (almost 6-fold over the past few decades) in the United States and Western Europe. Early diagnosis and management are vital in BE pathogenesis. Genomic changes occurring during BE progression hold a promise as effective biomarkers or therapeutic targets. We developed a novel in-vitro BE carcinogenesis (BEC) model by exposing hTERT immortalized benign Barrett's epithelium cells (BAR-T) to acid and bile at pH4 (B4), 5 min each day for 65 weeks (Das et.al., IJC 2010). Four phases highlight primary events in the BEC model (Figure 1): 1- **Phenotype change**- after two weeks of B4 exposure cell change to colonic (mAbDas-1+) phenotype or incomplete intestinal type metaplasia; 2: **Genomic changes** ~ 20wks of B4 exposure development of aneuploidy, changes in gene expression, methylation and mutation status; 3: **Morphological changes**- ~ 40 weeks of B4 exposure, change in cell shape from elongated to oval and characteristic clumping; 4: **Loss of contact inhibition/soft agar colony formation** ~ 60 weeks of B4 exposure suggesting neoplasia/transformation. In this study we identified gene networks based on differential patterns of expression that correlated with progressive transformation in this in-vitro Barrett's epithelial carcinogenesis model.

OBJECTIVE

To identify gene sets that may be associated with transformed phenotype in the *in-vitro* progressive Barrett's epithelial carcinogenesis model.

METHOD

FPKM RNA-seq data was obtained for 18560 genes from BEC-0W, BEC-20W, BEC-40W and BEC-60W cells. Expression pattern of each gene across the different time points were identified using the following statistical method: for each gene i, from the data vector X(i,j), with j=1,2,3,4 corresponding to control, 20, 40 and 60 wks respectively, we compute the mean expression across time points $\mu(i) = \Sigma_j X(i,j)/4$. We defined the expression level as high (=1), or low (= 0), relative to the mean (~ 99% confidence). Thus, the expression level of gene i is "high" if, $X(i,j) - a*\sqrt{X(i,j)} > \mu(i)$ and "low" if, $X(i,j) + a*\sqrt{X(i,j)} < \mu(i)$, where a=6. Using this criterion, we reduce the expression level X(i,j) to a vector V(i,j) which can take values 0, +/- 1. There are 14 states possible for V as listed below in Table 1:

Index	0	1	2	3	4	5	6	7
V(state)	(0,0,0,0)	(0,0,0,1)	(0,0,1,0)	(0,0,1,1)	(0,1,0,0)	(0,1,0,1)	(0,1,1,0)	(0,1,1,1)
Index	8	9	10	11	12	13	14	15
V(state)	(1,0,0,0)	(1,0,0,1)	(1,0,1,0)	(1,0,1,1)	(1,1,0,0)	(1,1,0,1)	(1,1,1,0)	(1,1,1,1)

Genes from four interesting sets were grouped into pathways using Ingenuity pathway analysis.

OBSERVATION

Four gene-sets (expression states: 1,3,8 and 12) shown in figure 2 a-d coincide with change of morphology in the BEC-40W and loss of adherence to substrate (colony formation) observed in BEC-60W cells. Pathway analysis revealed that genes from the four selected states are members of VEGF, RB, PTEN, ATF2, TP53, RAS, AKT, PI3K and other known oncogenic pathways.

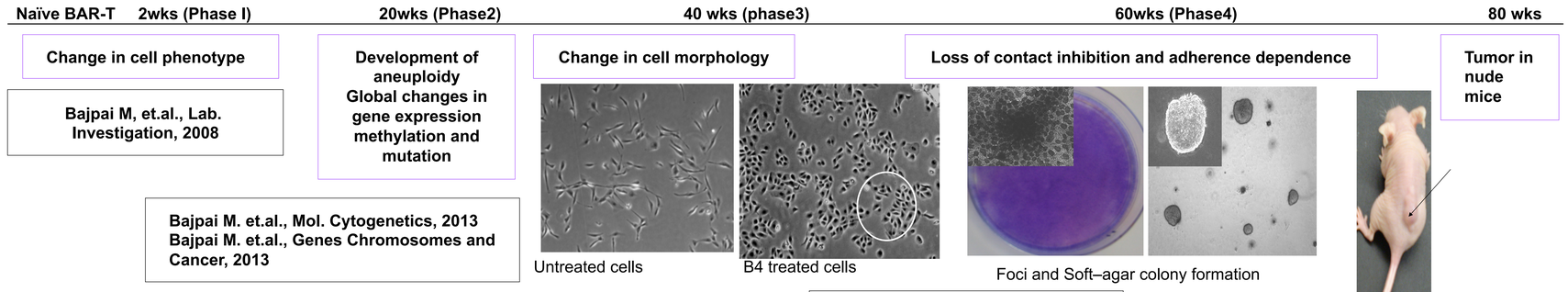

Fig 1: The Barrett's epithelial carcinogenesis model

Table 2: The number of genes in each of the four selected states (out of the 14 possible states). The expression state is denoted by binary digits 1=upregulation, 0=down regulation)

State #	Binary index of expression state (time in weeks=0,20,40,60)	Number of genes	
1	(0,0,0,1)	19	Fig:1a
3	(0,0,1,1,)	66	Fig:1b
8	(1,0,0,0)	18	Fig:1c
12	(1,1,0,0)	69	Fig:1d

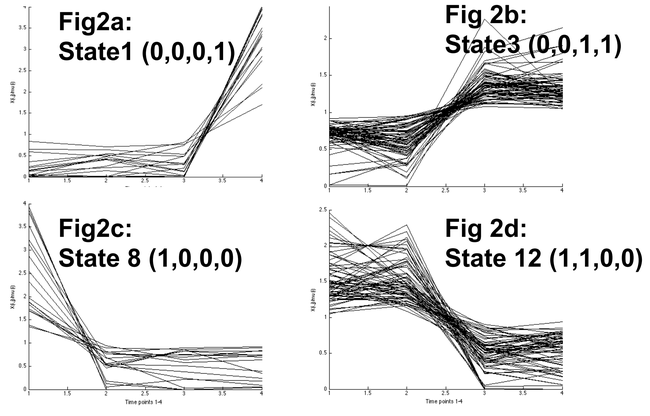

Fig 2 a,b,c, and d: graphical representation of four selected expression states with gene sets relevant to transformed phenotype in the BEC model

CONCLUSION

A statistical model identified four interesting gene sets from RNA sequencing data of the Barrett's epithelial carcinogenesis model. These gene sets comprise a short list of oncogenes, tumor suppressors as well as regulators of signal transduction that correlate with specific oncogenic pathways that may be responsible for the transformed phenotype observed in BEC-40W and BEC-60W cells.

Our observation from the BEC model corroborate that chronic exposure to B4 leads to genetic changes that can promote carcinogenesis in BE. Characterization of these potential candidate genes and pathways may lead to innovative biomarkers and therapeutic targets for early detection and management of potentially progressive Barrett's epithelium.