BMP Pathway Inhibitors May Prevent Neoplastic Progression of Barrett's Epithelium

Tu2055

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Introduction

The Inhibitor of DNA binding/ differentiation 2, (ID2) belongs to a family of helix-loop-helix (HLH) transcription factors that regulate cell proliferation and differentiation during embryogenesis. Expression of Id proteins is reactivated in lung, prostate, breast, bladder, colon and pancreatic cancers, and leads to increased proliferation and invasiveness. The ID2 activation is often associated with activation of the Bone morphogenetic protein (BMP) pathway and the possibility of inhibiting activated BMP and ID2 pathways using small molecular inhibitors (e.g., DMH2) of the BMP type 1 receptors (BMPR1A) have been studied extensively in lung cancer. The role of ID2 and BMP pathway in BE progression and esophageal adenocarcinoma (EA) is still unknown. 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). The BEC model was developed earlier after prolonged exposure of hTERT immortalized benign human BE cells, BAR-T, to acidified (pH4) and bile salt Glycocheno-deoxycholic acid, a physiological component of gastric refluxate (B4), 5min/day for a year. Progressive neoplastic changes accumulated e.g., amplification of colonic / intestinal phenotype mAbDas-1$^+$ and CK8$^+$ cells (Lab Invest. 2008), chromosomal aberrations (Mol. Cytogenet. 2012), change in cell shape with clumping soft agar colony formation 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. This BEC model has been found to recapitulate several genetic events reported by other investigators in human BE and EA tissues.

Hypothesis

ID2 overexpression is a potential mechanism of Barrett's carcinogenesis and can be prevented by DMH2

Methods

RNA sequencing of the BEC model cells at different time points of B4 exposure demonstrates intrinsic activation of several components of the BMP and ID2 pathway, in the BEC40W cells (exposed to B4 for 40 weeks) accompanied by increased proliferation and properties of malignant transformation. . ID2 inhibition with DMH2 was performed using a novel in-vitro BE carcinogenesis (BEC) model. The BEC40W cells were treated with 1uM and 5uM DMH2 for 24hrs and transcript levels of Id2, BMP2 and BMPR1A genes were measured. To confirm the clinical relevance of ID2 upregulation in EA, gene expression data on ID2 was collected from 89 esophageal adenocarcinoma tumors and 8 normal adjacent tissue available in The Cancer Genome Atlas - Data Portal (TCGA) and analyzed .

Table: Change in transcript levels of BMP pathway genes

Genes	Fold increase in BEC40W cells
BMPR1A	1.7
ID2	6.1
BMP2	7.2
ACVR1	1.4
ACVR2	1.5

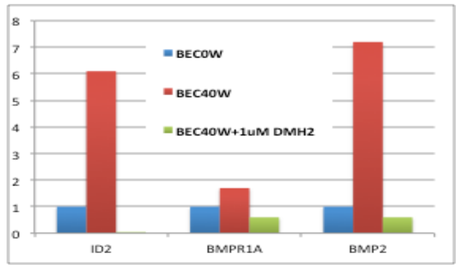

FIGURE 1: QRT- PCR reveals significant increase in transcript levels of ID2, BMP2 and BMPR1A genes in transformed BEC40W cells compared to control untreated BEC0W cells. ID2 and BMP2 are significantly suppressed by DMH2, a small molecular inhibitor of BMPR1A.

FIGURE 2: Significant overexpression of ID2 gene ($p<0.03$) was observed from RNA sequencing data on 89 esophageal adenocarcinoma tumors (EA, red) when compared to 8 normal adjacent tissues (NAT, green) available in the The Cancer Genome Atlas database.

Boxplots were created for ID2 expression in Tumor vs normal area and p-values were calculated using the rank sum test.

Results and Discussion

- Elevated transcript levels of ID2 (Inhibitor of DNA binding 2) (6.1 fold), BMP2 (7.2 fold) and BMPR1A (Bone Morphogenetic Pathway type 1 receptor) (1.7 fold) were observed in the BEC40W cells when compared to BEC0W cells (Figure 1). This increase in BMPR1A and ID2 was also accompanied by increased expression of other BMP family members (table).
- Intriguingly, bioinformatics analyses of TCGA database revealed significant overexpression of ID2 ($p< 0.03$) in EA tumors compared to the normal adjacent tissues (Figure 2). This observation provides evidence that ID2 upregulation is common in EA.
- When BEC40W were exposed to 1uM DMH2, ID2 expression was significantly reduced (0.01 fold) within 24hrs, some reduction of BMP2 (0.6 fold) (average of two experiments) and BMPR1A (0.6 fold only with 5uM DMH2) was also observed (Figure 1).

Conclusion

ID2, a gene that promotes cancer cell proliferation and metastasis, is amplified in EA and may be a potential therapeutic target for chemoprevention. In this report we demonstrate that the BEC model may be used as a novel tool to study the functional role of ID2, and small molecular inhibitor (DMH2) mediated inhibition of ID2 as a possible chemoprevention option in BE carcinogenesis.

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