

Discovery of the BMPR1A promoter and genetic alterations that may cause juvenile polyposis

*Daniel Calva MD, Sathivel Chinnathami PhD,
James R Howe MD, FACS*

Carver College of Medicine, The University of Iowa Hospitals and Clinics, Iowa City, IA

INTRODUCTION: Patients with juvenile polyposis (JP) develop colorectal and upper GI polyps and have a >50% risk of developing GI cancer. Mutations in SMAD4 and BMPR1A cause JP; however, only 45% of patients have identifiable mutations. The promoter of BMPR1A has not been described, and genetic alterations (GAs) here could lead to JP. The objective was to characterize the promoter of BMPR1A and to screen JP patients for GA.

METHODS: 5'RACE was used to identify potential transcriptional start sites, and Genomatix promoter prediction software used to identify candidate regions. Deletion constructs were cloned into luciferase vectors and tested for activity. MatInspector software identified potential regulatory binding sites, JP probands and controls were sequenced for GA, and ELISA was performed to determine protein levels of BMPR1A.

RESULTS: 5'RACE identified 4 noncoding exons. Two putative promoters were identified, with one having minimal luciferase activity (9 light units [lu] in HEK-293 cells normalized to controls), while the other had strong promoter activity (291 lu); this latter region was predicted to be a promoter by Genomatix software. From 54 JP probands, 15 had GA in this promoter, all predicted to be at regulatory binding sites. Five of these 15 were analyzed by ELISA and had substantially decreased BMPR1A protein.

CONCLUSIONS: We have identified the promoter for BMPR1A. Several JP patients without coding sequence mutations had GA in this region, which were not found in controls. The finding of low protein levels in these patients suggests that promoter mutations could lead to the development of JP.

Extracellular matrix 1 (ECM1) is overexpressed in anaplastic thyroid cancers and appears to be regulated by transcription factor AP2C (TFAP2C)

*Geeta Lal MD, MSc, Lakshmi Padmanabha BSc,
Piedad Gomez-Contreras PhD, Lurong Zhang MD, PhD,
George Woodfield MSc, Ryan W Askeland MD,
Ronald J Weigel MD, PhD, FACS*

University of Iowa, University of Rochester Medical Center, Iowa City, IA, and Rochester, NY

INTRODUCTION: ECM1 is overexpressed in and has been identified as a novel diagnostic and possible prognostic marker for differentiated thyroid cancer. Its regulation has not been studied. We hypothesized that (1) ECM1 is upregulated in anaplastic thyroid cancers and (2) increased ECM1 expression in thyroid carcinomas is mediated by TFAP2C (based on work in breast cancer).

METHODS: ECM1 and TFAP2C expression was examined in thyroid tumor cell lines and primary tumors using real-time RT-PCR, Western blotting, or immunohistochemistry. TFAP2C expression was blocked using small interfering RNA (siRNA) and upregulated using adenoviral AdAP2C recombinants.

RESULTS: ECM1 was overexpressed in 8/12 (66.7%) primary anaplastic thyroid cancers. Knockdown of TFAP2C expression in DRO-90 cells, which overexpress TFAP2C and ECM1, was associated with a concomitant significant reduction in ECM1 mRNA (mock vs TFAP2C, 1 vs 0.22; $p < 0.001$) and protein expression (to 20% of mock-transfected cells). Overexpression of TFAP2C in KAT4B cells, which did not express high levels of TFAP2C or ECM1, led to an increase in ECM1 mRNA and protein expression. 5'RACE for ECM1 identified a major transcription initiation site at -100 and a minor site at -150. Initial characterization of a 2-kb fragment proximal to the ECM1 start site cloned upstream of a luciferase reporter shows that it has promoter activity.

CONCLUSIONS: ECM1 is overexpressed in anaplastic thyroid cancers at a rate higher than that reported for differentiated thyroid tumors. Furthermore, TFAP2C appears to regulate ECM1 expression. Delineating the ECM1 promoter and the mechanism of regulation by TFAP2C may enable identification of novel therapeutic strategies for aggressive thyroid malignancies.

BRCA1 is an essential regulator of cardiac function

*Praphulla C Shukla PhD, Krishna K Singh PhD, Fina Lovren PhD,
Yi Pan MD, Howard Leong-Poi MD, Lee Errett MD,
William L Stanford PhD, Michael D Schneider MD,
Thomas G Parker MD, Subodh Verma MD, PhD*

St Michael's Hospital, University of Toronto, Imperial College London, Toronto, ON, Canada

INTRODUCTION: Preservation of structure and function of the myocardium is critically dependent upon improving the survival of existing cardiomyocytes (CMs) through strategies that limit CM apoptosis and DNA damage. BRCA1 is a tumor suppressor gene that promotes DNA repair and protects cells against oxidative and genotoxic stress. We hypothesized that BRCA1 is a novel target to limit CM apoptosis and prevent aberrant cardiac remodeling.

METHODS: Studies were performed on mice and CM.

RESULTS: Experimental MI in mice caused a 16-fold upregulation in BRCA1 expression, which peaked at 72 hours ($p < 0.01$). Ad-BRCA1 overexpression protected neonatal rat CM against doxorubicin- and H₂O₂-induced apoptosis ($p < 0.01$) and activated caspase-3. p53 expression, in response to doxorubicin and H₂O₂, was reduced in Ad-BRCA1-expressing CM. Co-immunoprecipitation studies demonstrated a physical interaction of BRCA1 with p53. Inhibition of p53, with pifithrin-alpha, blocked doxorubicin-induced CM apoptosis in a manner similar to BRCA1. BRCA1-overexpressing CM, when treated with doxorubicin did not show further reduction with pifithrin-alpha, indicating a requirement of BRCA1 to modulate p53. Systemic Ad-BRCA1 delivery prevented doxorubicin-induced cardiac dysfunction in mice (echocardiography; $p < 0.01$). CM-specific BRCA1-KO mice demonstrated marked cardiac dysfunction and mortality in response to doxorubicin ($p < 0.01$ vs WT + Dox).

CONCLUSIONS: We report for the first time an essential role of BRCA1 to limit CM apoptosis and improve cardiac function in response to genotoxic and oxidative stress. Heart-specific BRCA1 deletion promotes severe systolic dysfunction and limits survival.

Aside from implications for cardiovascular repair, these data may have ramifications for individuals with BRCA1 mutations, particularly in the setting of adjuvant chemotherapy.

Obesity perturbs the insulin axis and downregulates pancreatic cancer matrix genes

Kathryn M Dalbec MD, Hayder H Al-Azzawi MD, Debora Schwartz-Basil PhD, Gbenga Okusanya BA, Sue Wang MD, Yunlong Liu PhD, Henry A Pitt MD, FACS, Nicholas J Zyromski MD, FACS
Indiana University School of Medicine, Indianapolis, IN

INTRODUCTION: We have recently shown that obesity potentiates pancreatic cancer growth and dissemination in obese mice. The tumor matrix in obese mice differed significantly (greater adipocyte volume) from that in lean mice, and tumor proliferation correlated strongly with increased insulin concentration. As insulin axis dysregulation is known to influence pancreatic cancer growth and modulate matrix biology, we sought to identify differentially expressed genes in tumors from lean and obese mice.

METHODS: Murine pancreatic tumors (from PAN02 cells) were grown for 5 weeks in 6 lean (C57BL/6J), 6 obese leptin-deficient (LepOb), and 6 leptin-resistant (LepDb) mice. Differential tumor gene expression was measured using Illumina microarray chip technology (45,000 expressed sequence tags). A false discovery rate of 20% was applied, mean gene expression used for normalization, and the detection value threshold set at $p < 0.01$.

RESULTS: The larger tumors from obese mice differentially expressed 895 genes compared with tumors from lean mice. Seventeen genes related to the insulin axis were differentially expressed in obese tumors (LepOb, 4 upregulated, 11 downregulated; LepDb, 9 upregulated, 1 downregulated; 4 common genes all upregulated). Thirty-six matrix genes were differentially expressed and generally downregulated in obese tumors (LepOb, 1 upregulated, 26 downregulated; LepDb, 4 upregulated, 13 downregulated; common genes 1 upregulated, 11 downregulated). The table shows representative genes and degree of differential expression.

| Symbol | Db | Ob | Name |
|---------|-------|------|-----------------------------------------------------|
| Igf2bp1 | +10.5 | +8.4 | Insulin-like growth factor 2 mRNA binding protein 1 |
| Col4a2 | -1.7 | -1.7 | Collagen, type IV, alpha2 |
| Col4a1 | -1.7 | -1.7 | Collagen, type IV, alpha 1 |
| Col7a1 | -1.8 | -1.9 | Collagen, type VII, alpha 11 |
| Mmp11 | -1.9 | -1.9 | Matrix metalloproteinase 11 |
| Myo1b | -2.0 | -2.1 | Myosin 1B |
| Wnt7b | -2.4 | -2.4 | Wingless-related MMTV integration site 7b |
| Lgi2 | -3.3 | -3.2 | Leucine-rich repeat LGI family, member 2 |
| Tnc | -3.3 | -3.7 | Tenascin C |
| Abi3bp | -4.4 | -5.8 | ABI gene family, member 3 (NESH) binding protein |

CONCLUSIONS: These data show that obesity perturbs the insulin axis and downregulates pancreatic cancer matrix genes. Insulin dysregulation leading to release of the matrix "brake" may therefore be crucial in potentiating the growth of pancreatic cancer in obesity.

The role of APC gene mutation in breast carcinogenesis: Prevention by dietary curcumin

Ronald M Brooks MD, Tilda Barliya PhD, Noghma Wynne BS, Meena Katdare PhD
Weill Cornell Medical College, New York, NY

INTRODUCTION: Dysfunction of the APC gene, the most common genetic alteration in sporadic colorectal cancers, is also observed in 45% of sporadic breast cancers; however, its role as a risk factor for breast cancer remains unknown. This preclinical study was designed to evaluate ENU-induced mammary tumors in ApcMin/+ mice with defined risk for breast cancer and potential of dietary curcumin as a chemopreventive agent.

METHODS: 21-day-old ApcMin/+ and C57BL/6J mice randomized into 4 groups (normal diet-control, normal diet + ENU, 2% curcumin diet-control, 2% curcumin diet + ENU) were given single IP doses of either saline or ENU, 50 mg/kg, at 35 days of age. Mice were monitored for body weight, morbidity, and mammary tumors for 12 weeks. Tumor incidence, multiplicity, and size were recorded, and glands were processed for histopathology, APC/beta-catenin/cyclin-D1 mitogenic pathway by IHC/IF, and Western blot.

RESULTS: No loss in body weight was observed. No tumors were observed in C57BL/6J mice. In ApcMin/+ mice, tumor incidence was 20% in the normal diet-control group, while an 80% incidence was observed in the normal diet-ENU group. In the 2% curcumin diet + ENU group, tumor incidence was also 80%, but multiplicity and size were reduced. All tumors were squamous cell carcinomas. No tumors were observed in the 2% curcumin diet-control mice. Molecular evaluation of proliferation markers revealed upregulation of cyclin D1 and Ki-67 in normal diet + ENU group and reduced in the 2% curcumin + ENU group.

CONCLUSIONS: Our results demonstrate APC gene mutation increases susceptibility to ENU-induced mammary carcinogenesis. 2% curcumin has preventive efficacy toward mammary tumor growth and multiplicity in ApcMin/+ mice.

Genomic profiling of pathologic complete response in locally advanced rectal cancer

Isabelle Bedrosian MD, Qiang Hao, L Ding, Miguel Rodriguez-Bigas MD, John M Skibber MD, George J Chang MD, FACS, Michael D Story PhD
The University of Texas MD Anderson Cancer Center, Houston, TX

INTRODUCTION: Approximately 20% of patients with locally advanced rectal cancer (LARC) undergoing preoperative chemoradiation therapy achieve a pathologic complete response (pCR). These patients may be candidates for novel organ-preserving therapies.