Histone Deacetylase Inhibition Modulates E-Cadherin Expression and Suppresses Migration and Invasion of Anaplastic Thyroid Cancer Cells

Maria Grazia Catalano, Nicoletta Fortunati, Mariateresa Pugliese, Francesca Marano, Loredana Ortolova, Roberta Poli, Sofia Asili, Andrea Bandino, Nicola Palestini, Cristina Grange, Benedetta Bussolati, and Giuseppe Bocuzzi

Context: Anaplastic thyroid cancer cells are characterized by a mesenchymal phenotype, as revealed by spindle-shaped cells and absent or reduced levels of E-cadherin. Epigenetic silencing is considered one of the leading mechanisms of E-cadherin impairment, which causes the acquisition of the invasive and metastatic phenotype of anaplastic thyroid cancer.

Objectives: In this study we investigated the effects of histone deacetylase inhibition on E-cadherin expression, cell motility, and invasion in anaplastic thyroid cancer cell cultures.

Design: Three stabilized cell lines and primary cultures of anaplastic thyroid cancer were treated with various histone deacetylase inhibitors. After treatment, we evaluated histone acetylation by Western blotting and E-cadherin expression by real time RT-PCR. The proper localization of E-cadherin/β-catenin complex was assessed by immunofluorescence and Western blot. Transcription activity of β-catenin was measured by luciferase reporter gene and cyclin D1 expression. The effect on cell motility and invasion was studied both in vitro using scratch-wound and transwell invasion assays and in anaplastic thyroid carcinomas tumor xenografts in mice in vivo.

Results: Histone deacetylase inhibition induced the E-cadherin expression and the proper membrane localization of the E-cadherin/β-catenin complex, leading to reduced cancer cell migration and invasion.

Conclusions: We here demonstrate an additional molecular mechanism for the anticancer effect of histone deacetylase inhibition. The antiinvasive effect in addition to the cytotoxic activity of histone deacetylase inhibitors opens up therapeutic perspectives for the anaplastic thyroid tumor that does not respond to conventional therapy.

Somatostatin Analogs Modulate AIP in Somatotroph Adenomas: The Role of the ZAC1 Pathway


Context: Somatotroph adenomas harboring aryl hydrocarbon receptor interacting protein (AIP) mutations respond less well to somatostatin analogs, suggesting that the effects of somatostatin analogs may be mediated by AIP.

Objective: The objective of the investigation was to study the involvement of AIP in the mechanism of effect of somatostatin analogs.

Design: This was a human study, a 16-wk somatostatin analog pretreatment compared with no pretreatment. The cell line was somatostatin analog treatment or small interfering RNA (siRNA)/plasmid transfection compared with an appropriate control.

Setting: The study was conducted at a university hospital.

Patients: Thirty-nine sporadic and 10 familial acromegaly patients participated in the study.

Intervention: Interventions included reoperative lanreotide treatment and pituitary surgery.

Outcome: For the human study, GH and IGF-I levels, AIP, and somatostatin receptor staining were measured. For the cell line, AIP and ZAC1 expression, metabolic activity, and clone formation were measured.

Results: Lanreotide pretreatment reduced GH and IGF-I levels and tumor volume (all \(P < 0.0001\)). AIP immunostaining was stronger in the lanreotide-pretreated group vs. the surgery-only group (\(P < 0.001\)). After lanreotide pretreatment, the AIP score correlated to IGF-I changes in females (\(R = 0.68, P < 0.05\)). Somatostatin receptor staining was not reduced in samples with AIP mutations. In GH3 cells, 1 nM octreotide increased AIP mRNA and protein (both \(P < 0.01\)) and ZAC1 mRNA expression (\(P < 0.05\)). Overexpression of wild-type (but not mutant) AIP increased ZAC1 mRNA expression, whereas AIP siRNA knockdown reduced ZAC1 mRNA (both \(P < 0.05\)). The siRNA-mediated knockdown of AIP led to an increased metabolic activity and clonogenic ability of GH3 cells compared with cells transfected with a nontargeting control (both \(P < 0.001\)).

Conclusion: These results suggest that AIP may play a role in the mechanism of action of somatostatin analogs via ZAC1 in sporadic somatotroph tumors and may explain their lack of effectiveness in patients with AIP mutations.

Higher Circulating Sphingosine 1-Phosphate Levels Are Associated with Lower Bone Mineral Density and Higher Bone Resorption Marker in Humans


This article appears in The Journal of Clinical Endocrinology & Metabolism, published May 4, 2012, 10.1210/jc.2012-1111

The following abstracts from The Journal of Clinical Endocrinology & Metabolism have been selected by the editors of Endocrinology as being particularly relevant to readers interested in translational science.
Context: Several \textit{in vivo} and \textit{in vitro} studies suggest that sphingosine-1-phosphate (S1P) is known to act as a coupling factor, to stimulate osteoclastogenesis, to control the migration of osteoclast precursors between the blood and bone, and to stimulate the proliferation, migration, and survival of osteoblasts.

Objective: Using the determination of circulating S1P levels, we investigated which kinds of processes may be primarily affected by S1P in humans.

Design and Setting: This was a cross-sectional study conducted in two clinical units in Korea.

Participants: Men (n = 86), premenopausal women (n = 94), and postmenopausal women (n = 357) participated in the study.

Main Outcome Measures: We measured S1P levels and their relationships with bone mineral density, biochemical bone turnover markers, and uncoupling indices.

Results: S1P levels were significantly higher in the postmenopausal women than in the premenopausal women and men. High S1P concentrations were significantly associated with low bone mineral density values at some femur sites in the postmenopausal women (P = 0.015 to 0.049), at the lumbar spine in the premenopausal women (P = 0.017), and at all sites in men (P = 0.001 to 0.036) after adjustments with multiple covariates. S1P levels were positively correlated with bone resorption markers (P = 0.003 to 0.049), but not with formation markers in postmenopausal women. Higher S1P levels were associated with lower uncoupling indices (P = -0.001 to 0.048) in postmenopausal women.

Conclusion: These findings suggest that S1P may primarily affect bone resorption, resulting in bone loss.

\textit{This article appears in The Journal of Clinical Endocrinology \& Metabolism, published June 7, 2012, 10.1210/jc.2012-1044}

\textbf{Placental Expression of Peroxisome Proliferator-Activated Receptor \gamma (PPAR\gamma): Relation to Placental and Fetal Growth}

Marta Diaz, Judit Bassols, Abel Lopez-Bermejo, Maria Dolores Gómez-Roig, Francis de Zegher, and Lourdes Ibáñez

Background and Objective: The nuclear receptor peroxisome proliferator activated receptor \gamma (PPAR\gamma) contributes to placental development and thus to the maternofoetal transfer of oxygen and nutrients that allow for prenatal growth. We tested the hypothesis that placental PPAR\gamma expression relates to placental and fetal growth.

Design and Study Population: Placentas (n = 116) were collected at term delivery of singleton infants who were born small- (SGA), appropriate- (AGA), or large-for-gestational-age (LGA) (n = 32 SGA, 55 AGA, and 29 LGA). Placentas and newborns were weighed at birth. Real-time PCR was used to assess placental expression of PPAR\gamma as compared to the housekeeping gene GAPDH.

Results: PPAR\gamma expression in placentas from AGA and LGA infants was nearly 2-fold higher than in placentas from SGA infants. Placental PPAR\gamma expression associated positively to placental and/or fetal weight at birth, particularly within the SGA subpopulation (P = 0.001).

Conclusion: PPAR\gamma expression was found to be low in placentas of SGA fetuses and to associate positively to fetal and placental weights within this subpopulation.

\textit{This article appears in The Journal of Clinical Endocrinology \& Metabolism, 10.1210/jc.2012-1064}

\textbf{Epidermal Growth Factor Induces Human Oviductal Epithelial Cell Invasion by Down-Regulating E-Cadherin Expression}

Jung-Chien Cheng, Hsun-Ming Chang, and Peter C. K. Leung

Context: The loss of E-cadherin enhances cell invasiveness. There is increasing evidence that high-grade serous ovarian cancer may arise from oviductal epithelial cells rather than the ovarian surface epithelium. Despite the controversy over the cellular origins of this disease, the roles of epidermal growth factor (EGF) in human oviductal epithelial cells are largely unknown.

Objective: We examined whether EGF could induce oviductal epithelial cell invasion by its down-regulation of E-cadherin.

Methods: Matrigel-coated transwells were used for the invasion assay. Small interfering RNA was used to knock down the expression of EGF receptor (EGFR). Specific mRNA and protein levels were examined by quantitative RT-PCR and Western blot, respectively.

Results: The expression of Pax8 confirmed the secretory type of the cultured human oviductal epithelial cell line OE-E6/E7. EGFR was expressed in OE-E6/E7 cells, and treatment with EGF down-regulated E-cadherin expression. The effect of EGF on the down-regulation of E-cadherin was abolished by small interfering RNA-mediated depletion of EGFR. EGF treatment led to the activation of ERK1/2, p38, and Akt. Snail and Slug are transcriptional repressors of E-cadherin. Interestingly, our results show that EGF induced Slug but not Snail expression. Moreover, the inhibition of EGF-induced ERK1/2, p38, and Akt activation by pharmacological inhibitors attenuated EGF-induced Slug expression and the down-regulation of E-cadherin, as well as subsequent cell invasion.

Conclusions: EGF induces human oviductal epithelial cell invasion through the activation of ERK1/2, p38, and Akt, the up-regulation of Slug, and the down-regulation of E-cadherin.

\textit{This article appears in The Journal of Clinical Endocrinology \& Metabolism, published May 8, 2012, 10.1210/jc.2011-2751}

\textbf{Significance of IGFBP-4 in the Development of Fetal Growth Restriction}

Qing Qiu, Mike Bell, Xiaoyin Lu, Xiaojuan Yan, Marc Rodger, Mark Walker, Shi-Wu Wen, Shannon Bainbridge, Hongmei Wang, and Andree Gruslin

Background: Fetal growth restriction (FGR) is a leading cause of perinatal mortality and morbidity. Animal studies suggest dysregulation of IGF-binding protein (IGFBP)-4 is significant in the development of FGR, although human data are lacking. We postulated that IGFBP-4 is expressed at the maternal fetal interface and plays a role in regulating IGF bioavailability.
Thus, maternal serum levels of IGFBP-4 may be associated with complications of abnormal placental growth and development including FGR.

Methods: Circulating levels of IGFBP-4 and its protease, pregnancy-associated plasma protein-A (PAPP-A), were examined in healthy pregnancies. Their expression in villi and bed as possible sources of the circulating products were examined by immunohistochemistry. From the large Ottawa and Kingston (Oak) Birth Cohort, a nested case-control study was conducted to examine circulating levels of IGFBP-4, PAPP-A, IGF-I, and IGF-II by Western blot in early gestation in 36 women who went on to develop FGR and 36 controls having normal-weight babies.

Results: IGFBP-4 was elevated in early pregnancy compared with nonpregnant women and women in later pregnancy, consistent with the presence of abundant extravillous trophoblasts and decidual cells that highly expressed IGFBP-4. High expression of PAPP-A was observed in extravillous trophoblasts and decidual cells in early pregnancy but hardly detectable in the circulation at this time, suggesting maternal circulating PAPP-A originates more likely from syncytiotrophoblasts. Increased IGFBP-4 in the maternal circulation in early pregnancy was associated with the development of FGR [0.48 (0.28-0.74) in control vs. 1.22 (0.66-1.65) in FGR; odds ratio = 22 (95% confidence interval = 2.7-181)]. No difference was observed in circulating PAPP-A, IGF-I and IGF-II in the FGR vs. control group.

Conclusion: Our findings support the role of IGFBP-4 in regulating IGF bioavailability and provide new clues for the prevention and treatment of FGR, raising the possibility of clinical use of IGFBP-4 as an early biomarker for this condition.

Lipid Peroxidation Is Associated with the Severity of Periodontal Disease and Local Inflammatory Markers in Patients with Type 2 Diabetes

Alliny de Souza Bastos, Dana T. Graves, Ana Paula de Melo Loureiro, Carlos Rossa Júnior, Dulcínéia Saes Parra Abdalla, Tanize do Espírito Santo Faulin, Niels Olsen Câmara, Oelsooa M. Andriankaja, and Silvana Regina Perez Orrio

Context: Periodontitis is the most common lytic disease of bone and is recognized as a common complication of diabetes. Lipid peroxidation (LPO) is increased in diabetes and may be related to modulation of the inflammatory response. LPO levels in patients with diabetes and periodontal disease have not been evaluated.

Objective: The aim of this study was to evaluate the levels of LPO and its correlation with periodontal status and inflammatory cytokines in type 2 diabetic and nondiabetic patients.

Design and Setting: This is a cross-sectional study involving Brazilian patients recruited at the State University of São Paulo.

Participants: The sample comprised 120 patients divided into four groups based upon diabetic and dyslipidemic status: poorly controlled diabetics with dyslipidemia, well-controlled diabetics with dyslipidemia, normoglycemic individuals with dyslipidemia, and healthy individuals.

Main Outcome Measures: Blood analyses were carried out for fasting plasma glucose, glycated hemoglobin, and lipid profile. Periodontal examinations were performed, and gingival crevicular fluid was collected. LPO levels were evaluated by measuring oxidized low-density lipoprotein (ELISA) and malondialdehyde (HPLC). Cytokines were evaluated by the multiplex bead technique.

Results: LPO evaluated by malondialdehyde in plasma and gingival crevicular fluid was significantly increased in diabetes groups. Significant correlations between LPO markers and periodontal parameters indicate a direct relationship between these levels and the severity of inflammation and secretion of inflammatory cytokines, particularly in diabetic patients.

Conclusion: These findings suggest an important association for LPO with the severity of the local inflammatory response to bacteria and the susceptibility to periodontal disease in diabetic patients.

This article appears in The Journal of Clinical Endocrinology & Metabolism, published May 7, 2012, 10.1210/jc.2011-3397

Metabolic and Neuroendocrine Responses to Roux-en-Y Gastric Bypass. I: Energy Balance, Metabolic Changes, and Fat Loss

X. Liu, A. Lagoy, I. Discenza, G. Papineau, E. Lewis, G. Braden, J. Romanelli, B. Braun, and J. E. Silva

Context: Obesity is a major health problem. Effective treatment requires understanding the homeostatic responses to caloric restriction.

Objective: The aim was to study Roux-en-Y gastric bypass patients longitudinally for 6 months after surgery to identify major factors modulating fat loss.

Methods: We studied 13 patients (11 females and two males) aged 41.2 ± 2 yr. Mean body mass index was 44.6 ± 1.2 kg/m², with 50 ± 1% body fat (58.3 kg). Selection excluded patients with confounding comorbidities or treatments.

Results: Caloric intake was reduced 742 ± 82 kcal/d by 1 month and 450 kcal/d between 2 and 4 months postoperatively. By 6 months, relative to baseline, body mass index decreased 24.8 ± 1.1%; percentage body fat, 37.3 ± 3.2% (21.7 kg); fat free mass (FFM), 9.7 ± 1.2%; and resting metabolic rate (RMR), 18.1 ± 4.3%. RMR correlated with FFM at all times (r = 0.71; P < 0.0001), but FFM explained no more than 50% of RMR variance. Exercise capacity (treadmill walking, 53 m/min with increasing grade) improved with time. Mean nonexercise physical activity level was low (1.2, or 20% of RMR), with considerable variance among individuals. Fat loss did not correlate with the aggregate energy deficit or its individual components. Resting or postexercise respiratory exchange ratio (RER) was lowest, whereas plasma β-OH-butyrate and glycerol were highest, between 1 and 2 months after surgery. RER increased linearly with mild exercise, and fat loss correlated positively with physical activity level and RER.

Conclusions: Although the ultimate cause for weight loss is the energy deficit, the variance in fat loss correlated with glucose oxidation, suggesting that glucose partition between oxidation (muscle) and storage (adipose tissue) is an important

This article appears in The Journal of Clinical Endocrinology & Metabolism, published May 14, 2012, 10.1210/jc.2012-1016

Adolescent Fiber Consumption Is Associated with Visceral Fat and Inflammatory Markers
Samip Parikh, Norman K. Pollock, Jigar Bhagatwala, De-Huang Guo, Bernard Gutin, Haidong Zhu, and Yanbin Dong

Context: The link between adolescent fiber consumption, inflammation, and body fat distribution has not been investigated.

Objective: This study investigated associations of dietary fiber intake with inflammatory-related biomarkers and robust measures of total and central adiposity in a sample of 559 adolescents aged 14–18 yr (49% female, 45% Black).

Methods: Fasting blood samples were measured for leptin, adiponectin, resistin, C-reactive protein, and fibrinogen. Diet intake was assessed with four to seven 24-h recalls, and physical activity was determined by accelerometer. Fat-free soft tissue mass and fat mass were measured by dual-energy x-ray absorptiometry. Visceral adipose tissue was assessed using magnetic resonance imaging.

Results: Multiple linear regression, adjusting for age, race, Tanner stage, fat-free soft tissue mass, energy intake, and physical activity, revealed that dietary fiber intake was inversely associated with fat mass and serum leptin in males (all P < 0.03) but not in females. In both genders, dietary fiber intake was negatively associated with visceral adipose tissue, plasma C-reactive protein, and plasma fibrinogen and positively associated with plasma adiponectin (all P < 0.05). No relations were found between dietary fiber intake and plasma resistin in either males or females.

Conclusion: Our adolescent data suggest that greater consumption of dietary fiber is associated with lower visceral adiposity and multiple biomarkers implicated in inflammation.

This article appears in The Journal of Clinical Endocrinology & Metabolism, published May 16, 2012, 10.1210/jc.2012-1784

ZNF764 Haploinsufficiency May Explain Partial Glucocorticoid, Androgen, and Thyroid Hormone Resistance Associated with 16p11.2 Microdeletion
Tomoshige Kino, Maria G. Pavlatou, Andreas G. Moraitis, Robin L. Nemery, Margarita Raygada, and Constantine A. Stratakis

Context: Nuclear hormone receptors exert their transcriptional effects through shared cofactor molecules; thus, defects in such intermediate proteins may be associated with multiple hormone resistance. Microdeletion of small chromosomal segments results in hereditary or sporadic diseases by affecting expression of residing genes.

Objectives: We describe a 7-yr-old boy with partial resistance to glucocorticoids, thyroid hormones, and possibly androgens. He was diagnosed as being in the autism spectrum disorder and had developmental delay and several facial morphological manifestations. We explored genes responsive to multiple hormone resistance of this case.

Results: We found in this patient an approximately 1.1-Mb heterozygous 16p11.2 microdeletion, which had an approximately 0.5-Mb extension in addition to a common approximately 600-kb 16p11.2 microdeletion. The small interfering RNA-based screening revealed that knockdown of ZNF764, which is located in the deleted segment unique to our case, significantly reduced glucocorticoid-, androgen-, and thyroid hormone-induced transcriptional activity of their responsive genes in HeLa cells, whereas its overexpression enhanced their transcriptional activity. The activities of the estrogen receptor, cAMP response element-binding protein, and p53 were not affected in these cells. ZNF764 expression was reduced in the patient’s peripheral blood mononuclear cells, whereas exogenously supplemented ZNF764 recovered responsiveness to glucocorticoids in the patient’s Epstein-Barr virus-transformed lymphocytes. The effect of ZNF764 on GR transcriptional activity was mediated through cooperation with a general nuclear hormone receptor coactivator, transcriptional intermediary factor 1.

Conclusions: ZNF764 haploinsufficiency caused by microdeletion may be responsible for the partial multiple hormone resistance observed in our patient. ZNF764 appears to be involved in glucocorticoid, androgen, and thyroid hormone action.

This article appears in The Journal of Clinical Endocrinology & Metabolism, published May 10, 2012, 10.1210/jc.2011-3493

Thyroid Dysfunction during Late Gestation Is Associated with Excessive Iodine Intake in Pregnant Women
ZhongNa Sang, Wei Wei, Na Zhao, GuiQin Zhang, Wen Chen, Hua Liu, Jun Shen, JiaYu Liu, YuQin Yan, and WanQi Zhang

Context: Adequate iodine intake during pregnancy is essential for both the synthesis of maternal thyroid hormones and the maintenance of normal fetal brain development. Scant evidence is available on the effects of excessive iodine intake during pregnancy.

Objective: The study assesses the relationship between iodine nutritional status and thyroid function of pregnant women with excessive iodine intake during late gestation.

Design and Participants: A cross-sectional study of 384 pregnant women was carried out in Tianjin and Haixing from April to October in 2010.

Main Outcome Measures: Morning urine samples and blood samples were obtained from all subjects. Serum levels of free T3, free T4, and sensitive TSH and urinary iodine concentration were measured.

Results: The median urinary iodine concentration of pregnant women with excessive iodine intake was significantly higher than those with adequate iodine intake (P < 0.001). The prevalence of thyroid disease, especially subclinical hypothyroidism, in pregnant women with excessive iodine intake was significantly higher than in those with adequate iodine intake (P < 0.05). Subclinical hypothyroidism was the most frequent pattern of thyroid disease for pregnant women and those with positive or negative thyroid autoantibodies. Living with high
water iodine content and having urinary iodine concentration higher than 250 μg/liter are associated risk factors for subclinical hypothyroidism in pregnant women (OR₁ = 41.822, OR₂ = 6.202; P < 0.05).

Conclusions: Excessive iodine intake during late pregnancy may lead to maternal thyroid dysfunction, particularly subclinical hypothyroidism. The appropriate measurements should be performed to monitor the onset of hypothyroidism in pregnant women with excessive iodine intake.

This article appears in The Journal of Clinical Endocrinology & Metabolism, published June 5, 2012, 10.1210/jc.2011-3438

Kisspeptin Administration to Women: A Window into Endogenous Kisspeptin Secretion and GnRH Responsiveness across the Menstrual Cycle

Yee-Ming Chan, James P. Butler, Valerie F. Sidhoum, Nancy E. Pinnell, and Stephanie B. Seminara

Context: Kisspeptin is the most powerful known stimulus of GnRH-induced LH secretion across mammalian species. However, the effects of kisspeptin are just being explored, and the dynamics of kisspeptin responsiveness across the menstrual cycle are incompletely understood.

Objective: The objective of the study was to characterize the effects of kisspeptin on GnRH secretion in healthy women in different phases of the menstrual cycle.

Participants and Intervention: Ten women in the early follicular phase, three women in the preovulatory phase, and 14 women in the midluteal phase received a bolus of kisspeptin 112-121 0.24 nmol/kg iv. An additional four women in the early to midfollicular phase received kisspeptin 112-121 0.72 nmol/kg iv.

Results: The response to kisspeptin varied, depending on the phase of the menstrual cycle. LH pulses were observed immediately after kisspeptin administration in all women in the luteal and preovulatory phases. However, only half the women in the early follicular phase had unambiguous kisspeptin responses. Increasing the kisspeptin dose did not increase the LH response in early to midfollicular phase women. Kisspeptin did not appear to reset the GnRH pulse generator in women as it does in men.

Conclusions: Differences in response to exogenous kisspeptin across the menstrual cycle suggest that kisspeptin tone is higher in the early follicular phase compared with other cycle phases. The mechanisms that determine the timing of GnRH pulse generation in men and women appear to be distinct.

This article appears in The Journal of Clinical Endocrinology & Metabolism, published May 10, 2012, 10.1210/jc.2012-1282