of *H. pylori* negative and positive volunteers were assessed by western blot and immunohistochemistry for angiotensin II receptors (AT1R, AT2R) and other RAS components (angiotensinogen, renin, angiotensin converting enzyme and neprilysin). Mucosa levels of myeloperoxidase (MPO) served as protein markers of neutrophil infiltration. Results: The AT1R protein expression was significantly higher in both antral and corporal specimens from *H. pylori* positive subjects compared to specimens from *H. pylori* negatives. The gastric mucosal AT1R protein expression correlated with mucosa levels of myeloperoxidase. No significant differences or correlations were found with regard to expression of the other investigated RAS components. Confocal microscopy showed that AT1R was highly expressed by a subpopulation of antral endocrine cells. Most other epithelial cells, as well as all leukocytes infiltrating the *H. pylori*-infected mucosa, also expressed AT1R and AT2R. Angiotensinogen and renin were expressed by resident mesenchymal cells in the lamina propria and all investigated RAS components were found in vascular endothelial cells. Conclusions: The results suggest that AT1R in the *H. pylori* infected gastric mucosa might influence neutrophil ROS generation and hormonal release from gastric endocrine cells. These mechanisms might be important for the development of H pylori induced peptic ulcers and gastric adenocarcinoma. This study also demonstrates local expression of several other RAS components in the human gastric mucosa that all might serve as pharmacological targets for modulation of RAS in the *H. pylori* infected gastric mucosa.

S1664

Glutamate Suppresses *Helicobacter pylori*-Induced Gastric Atrophy in Rodents - Mechanisms of Chief Cell Protection

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Backgrounds & Aims: Glutamate is the most abundant amino acid that composes body proteins. In the oral cavity, glutamate evokes umami taste, which is one of the five basic tastes perceived on the tongue, contributing to the preference and the palatability of the foods. In the GI tract, luminal free glutamate induces gastroduodenal mucus secretion and activation of the gastric afferent vagus nerve. Glutamate also works as an energy source in the GI tract during absorption. These findings imply the important roles of glutamate in the regulation of physiological GI functions such as food intake, digestion and absorption. Chronic *Helicobacter pylori* (HP) infection induces gastric diseases such as gastritis, gastric ulcer and gastric cancer, which worsen or negatively influence food intake, resulting in the loss of health. We have previously reported that Glu supplemented in the diet reduces HP-induced gastric mucosal damages in gerbils. Surprisingly, gastric atrophy (i.e. the loss of pepsinogen-secreting chief cells and acid-secreting parietal cells) was also reduced by Glu supplementation. To clarify the mechanisms how Glu reduces gastric atrophy against HP, we investigated the direct protective role of Glu and the surrounding metabolic pathways on the cell viability of cultured chief cells against ammonia, a known HP toxin. Methods: Gastric chief cell fractions were prepared from rats and gerbils using a counterflow elutriation and a density gradient centrifugation. We identified chief cell fraction by real-time RT-PCR using specific markers for each cell type. After 2 days culture, chief cells were treated with or without reagent(s) and/or NH_4Cl, then used for the following analysis such as cell viability and real-time RT-PCR. Results & Discussion: NH_4Cl, which mimics ammonia concentration in the gastric juice of HP-infected patients, significantly reduced cell viability. Glu, and the surrounding metabolites such as glutamine, asparagine and aspartate all protected cultured chief cells against NH_4Cl. Pharmacological analysis revealed that Glu synthetase inhibitors, L-methionine sulfoximine and L-2-amino adipic acid protected chief cells against NH_4Cl-induced cell death. In contrast, glutathione synthetase inhibitor, L-buthionine sulfoximine had no effect on Glu-induced chief cell protection against NH_4Cl. These results suggest that Glu reduces HP-induced gastric atrophy partly via glutamine synthetase-mediated pathway. Thus, we propose that free Glu in the diet would contribute to the protection from gastric atrophy against HP in daily life to keep healthy.

S1665

New Type IV Secretion System Homologue: *dupA* Gene Cluster of *Helicobacter pylori*

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Introduction: A number of *Helicobacter pylori* virulence factors have proven to be predictors of severe clinical outcomes of the infection. The duodenal ulcer promoting (*dupA*) gene of *H. pylori* was proposed to differentiation between duodenal ulcer (DU) and gastric cancer. Last year, we reported evidence that the *dupA* gene may be a component of a new gene cluster encoding a type IV secretion system. The aims of this study were to investigate whether *vir* gene homologues are located in the novel *dupA* gene cluster and to test the association of the *dupA* cluster with the clinical outcome in the US population. Methods: *H. pylori* strains were obtained from the gastric mucosa of 206 patients who underwent endoscopy at the Veterans Affairs Medical Center, Houston. To investigate the presence or absence of *vir* genes of *dupA* cluster, *vir* gene homologues (*dupA, virB8, B9, B10, B11, D4* and *D2*) were genotyped using PCR based methods. The association between genotype status and clinical outcomes were analyzed. Results: The prevalence of the *dupA* gene was 71.8%. The *vir* gene homologues were: 67.3% for *virB8*, 61.7% for *virB9*, 57.8% for *virB10*, 60.7% for *virB11*, 70.4% for *virD4* and 66% for *virD2*. The prevalence of each *vir* gene homologues in the *dupA* cluster was significantly higher in *dupA* positive strains than in *dupA* negative strains (P<0.001). There were no clinical correlation of each *vir* gene with diseases. The presence of an intact *dupA* cluster (i.e., *dupA, virB8, B9, B10, B11, D4* and *D2* were all positive) showed a tendency towards higher association with DU compared with gastritis (P=0.086). Conclusion: The *dupA* gene is a component of a new *H. pylori* gene cluster hypothesized to encode a type IV secretion system. Further studies of strains contain a complete component, including *dupA*, is required to identify it role as a secretion system and how it is involved in the pathogenesis of gastroduodenal disease.

S1666

Involvement of DNA Methyltransferase Expression in *H. pylori* cagA Induces Hypermethylation of Let-7 and Upregulation of RAS in Gastric Epithelial Cells

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<Background&Aim>Infection with CagA positive *H. pylori* is considered to be a risk factor for the development of gastric cancer. However its carcinogenesis remains unclear. We investigated the effect of CagA expression on micro(mi)RNA expression in gastric epithelial cells by miRNA array *In Vitro*, and revealed that let-7 was detected as one of the miRNAs whose expression was most significantly affected by CagA expression. Many miRNAs are regulated by epigenetic systems. One of epigenetic systems, DNA methylation, is induced by DNA methyltransferase (DNMT), and DNMT3A and 3B are responsible for establishing new DNA methylation. The aim of the present study was to determine the effect of CagA on expression of let-7 and its target molecules in gastric epithelial cells, and mechanism by which CagA suppressed let-7 expression. <Method> For *In Vivo* study, we constructed CagA transgenic mice of which cagA expression was regulated by tetracycline (Tet)-off system. Ras and let-7 expression in the stomach were compared between Tet-on and -off mice. To investigate the precise mechanism, cells were established by transfecting cagA genes into non-transformed rat gastric mucosal cultured cell line (RGM-1) cells using stable transfection of expression vectors containing Tet-off system. The effect of the replenishment of decreasing let-7 expression was investigated by let-7 precursor transfection. Promoter CpG island methylation of rat let-7 was ascertained by a PCR based methylation sensitive restriction enzyme assay, and DNMT1, 3a, and 3b expression were investigated by RT-PCR. <Result> In CagA transgenic mice study, CagA expressing stomachs revealed significantly decreased let-7 and enhanced Ras expression compared with control stomach. In *In Vitro* study, it was confirmed that CagA significantly reduced let-7 miRNA expression, and induced Ras expression. Let-7 precursor restored accelerated Ras expression in CagA expressing cells. Demethylating agents led to recovery of let-7 expression and suppress of Ras expression. CpG methylation analysis showed that CagA expression induced hypermethylation of rat let-7 promoter region. DNMT3b expression was enhanced in CagA expressing cells comparing with control, whereas no significant difference in DNMT1 or DNMT3a expression was detected in between control and CagA expressing cells. <Conclusion> The results indicate that CagA is involved in let-7 downregulation through hypermethylation due to DNMT3b induction, leading to its downstream Ras upregulation, which is suggesting that let-7 downregulation might account for CagA-related ERK activation, resulting in CagA related gastric carcinogenesis.

S1667

Transcriptional Suppression of STAT3 by TGF-β Signaling in Hepatocellular Cancer

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Hepatocellular cancers (HCC) may develop from malignant transformation of liver progenitor/stem cells. Several signaling pathways, including IL-6/STAT3 and TGF-β are known to be involved in stem cell renewal, differentiation and survival, and are commonly deregulated in HCC. We have previously demonstrated that human and mouse HCC tissues with aberrant TGF-β signaling show increased expression of STAT3. Moreover, down-regulation of the IL-6/STAT3 pathway in a TGF-β disrupted mouse model (β2SP+/-) by crossing with itih4–/– (Inter-alpha-trypsin inhibitor-heavy chain-4) mice resulted in a significant decrease in the incidence of HCC. Purpose: This led us to hypothesize that the TGF-β signaling pathway is a strong candidate pathway for the transition from progenitor to differentiated cells and its disruption may activate the stem cell renewal IL-6/STAT3 pathway. We proceeded to investigate the role of key TGF-β signaling components, including the key Smad3/4 adaptor protein β2SP and Smad3, in the regulation of STAT3 expression. Experimental procedures and Results: First, using early passage mouse embryonic fibroblasts (MEFs) from wild type and β2SP-/- embryos, we demonstrate a four-fold increase in STAT3 mRNA by RT-PCR (p<0.001). Moreover, we demonstrate that overexpression of β2SP by transfection or inhibition of β2SP expression by siRNA in several HCC cell lines results in STAT3 suppression or induction, respectively, suggesting that β2SP and the TGF-β signaling pathway regulate STAT3 transcription. Then, using ChiP assay, we demonstrate that β2SP and Smad3 are bound to the STAT3 promoter only following TGF-β stimulation. To then further define the molecular mechanism of TGF-β-mediated STAT3 transcriptional regulation, we then used mutational analysis and demonstrate two transcription factor binding sites within the STAT3 promoter, the cAMP-responsive element (CRE) and STAT3 binding (SBE) sites, are essential for TGF-β-mediated regulation of the STAT3 transcription. Subsequent, electrophoresis mobility shift assays (EMSA) demonstrate that the CRE-binding protein ATF-2, Smad3, and β2SP proteins are major components of the TGF-β-mediated STAT3 transcriptional suppressor complex. Conclusion: These experiments demonstrate a clear link between the TGF-β and IL-6/STAT3 signaling pathways. TGF-β suppression of STAT3 transcription is mediated by a complex including β2SP, Smad3 and ATF-2 at the CRE site of the STAT3 promoter. Inactivation of TGF-β signaling via disruption of β2SP decreases STAT3 suppression and suggests a potential mechanism for malignant transformation in TGF-β deficient progenitor/stem cells.

S1668

The PDZ Proteins, MAGI-3 and NHERF2, Reciprocally Regulate the Oncogenic Effects of LPA2 Receptor

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Lysophosphatidic receptor type 2 (LPA2) is a prototypic G protein-coupled receptor (GPCR) that interacts with multiple PSD-95/DlgA/ZO-1 (PDZ) proteins. In this study, we investigated functional modulation of LPA2 by two PDZ proteins, Na+/H+ exchanger regulator factor 2 (NHERF2) and membrane-associated guanylate kinase with inverted orientation-3 (MAGI-3). Previous studies showed that NHERF2 enhances LPA2-evoked signaling, but the role of