P113-Ab0098

Helicobacter Pylori-induced STAT3 Activation and Signalling Network in Gastric Cancer

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Background: Helicobacter pylori (H. pylori) is the most important gastric carcinogen. However, the mechanisms of H. pylori induced gastric carcinogenesis through STAT3 activation are largely unknown. We evaluated the effects of H. pylori infection on STAT3 activation and dissected the signalling network of STAT3 in H. pylori-infected gastric carcinogenesis.

Methods: The expression of phospho-STAT3 (pSTAT3) was evaluated by immunohistochemistry and western blot. Gene expression array and chromatin immunoprecipitation were used to dissect the STAT3 signalling network on H. pylori co-cultured AGS.

Results: The STAT3 activation (pSTAT3) was significantly higher in H. pylori-positive gastritis (73.5%, 61/83) than in H. pylori-negative gastritis (50%, 35/70) ($P = 0.003$). In addition, 98% (43/44) of H. pylori positive intestinal metaplasia specimens showed STAT3 activation, whereas pSTAT3 was significantly decreased in all 43 specimens one year after H. pylori eradication ($P < 0.001$). Moreover, pSTAT3 was only detected in the H. pylori-infected gastric tissues of mice but not in control mice, providing direct evidence that H. pylori infection stimulated the activation of STAT3 in the stomach. By comparing the gene expression profiles, we identified a total of 849 aberrant expression genes enriched in 11 cancer pathways in H. pylori induced STAT3 activation in gastric cancer cells. We further identified 6 candidates (BRUNOL4, FGFR1, SHOX2, JAK3, MAPK8, and PDPN) were directly up-regulated by H. pylori induced STAT3 activation.

Conclusions: H. pylori infection triggers the activation of STAT3 and de-regulates multitude of tumorigenic genes which may contribute to the initiation and progression of gastric cancer.

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P114-Ab0133

Role of Toll-like Receptors in Naturally Occurring Influenza

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Background: We investigated the roles of Toll-like receptors (TLRs) in naturally occurring influenza.

Methods: A prospective, case-control study was conducted. Adults hospitalized with virologically confirmed influenza A infections (onset <48 hours, before treatment) were compared with age-/gender-matched controls. TLRs (2, 3, 4, 7, 8, 9) expression in monocytes and dendritic cells (DCs - total, myeloid, plasmacytoid) was quantitated using flow cytometry. Gene expression of RLRs (RIG-1, MDA-5) was evaluated using real-time PCR. Concomitant signaling molecules expression, plasma cytokine/chemokine concentrations, and respiratory tract viral loads were measured. PBMCs were cultured and stimulated ex vivo with TLR-specific ligands for cytokine responses.

Results: Forty two patients with influenza (24 A/H3N2, 18 A/H1N1pdm09) and 20 controls were studied. Patients' mean age was 68 16 years; 81% had respiratory/cardiovascular complications. There were increased cellular expressions of TLR9, TLR8, TLR3, and TLR7 during influenza; TLR2 and TLR4 were suppressed. Results were similar for both virus strains. Higher TLR expression levels at presentation significantly correlated with lower viral loads (Spearman's rho: 046 to 069 for TLR9, TLR8, and TLR3; P-values <005). Multivariate regression models (adjusted for age, comorbidity, disease severity, time from onset) confirmed their independent associations. Increased signaling molecules (phospho-MAPKs, IkB) and inflammatory cytokines (IL-6, sTNFR-1, CCL2/MCP-1; CXCL10/IP-10, IFN-c) correlated with increased TLR expression. RLRs were upregulated simultaneously. PBMCs of patients with influenza showed significant, dynamic changes in their cytokine responses upon TLR stimulation, compared with controls.

Conclusions: Our results suggest that TLRs play an important role in early, innate viral inhibition in naturally occurring influenza. Inflammatory cytokine responses are concomitantly induced. These findings support investigation of TLR targeting as a novel intervention approach for prophylaxis against influenza.

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P115-Ab0101

Prevention and Treatment of Swine Origin Influenza Virus (S-OIV) through the Use of Interferon: An in vivo and ex vivo Study

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Background: H1N1pdm infection emerged in 2009 as the first influenza pandemic of the 21st century and originated from a swine precursor virus. Though this new virus was associated with a low mortality compared with seasonal influenza and with another new emerging influenza virus (H5N1), the main concerns that exist were the mortality in younger patients (contrasting with a bias towards elderly patients with seasonal influenza) and the potential for this virus to re-assort with avian influenza viruses resulting in a highly transmissible virus with a high mortality.

Objectives: To determine if prophylactic and/or therapeutic administered interferon alpha to ex vivo bronchial and lung tissues would be able to reduce H1N1pdm influenza infection.

Methods: Fresh bronchial and lung tissues were obtained from patients undergoing elective surgery with lobectomy. They were cultured with and without interferon and then infected with H1N1pdm virus. After 24 and 48 hours incubation the quantity of virus produced and the cellular genes involved in the antiviral response were analyzed by PCR and by SuperArray.

Results: Prophylactic interferon was able to reduce infection with H1N1pdm in lung tissues. Therapeutic interferon was beneficial in lung tissues but not in bronchial tissues.

Conclusions: Exogenous interferon will be useful for pulmonary involvement of influenza viruses (H1N1 and H5N1) but may not be of significant benefit for bronchial infection.

Implications: For influenza virus infections that are resistant to currently available antiviral agents, interferon therapy offers a potential benefit. This therapy has recently been used in a macaque model of H5N1 infection resulting in a decrease in the

alveolar inflammatory response.

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P116-Ab0102

Profiling of Substrate-Specificity and Rational Design of Broad-Spectrum Peptidomimetic Inhibitors for the 3C-Like Proteases of Coronaviruses

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Coronaviruses (CoV) are associated with up to 5% of respiratory diseases. There are three groups of CoV, namely alphacoronavirus (group 1), betacoronavirus (group 2), and gammacoronavirus (group 3). Replication of CoV requires an active 3C-like protease that processes the viral polyprotein into mature functional proteins. Supported by a HMRF grant, our group has profiled the substrate specificity of the 3C-like proteases from human CoV NL63 (group 1), human CoV OC43 (group 2a), SARS-CoV (group 2b), and infectious bronchitis virus (IBV; group 3). Based on the substrate specificity profile obtained, we have designed a 'super-active' substrate with ~2-3 fold increase in cleavage efficiency. We also designed a number of nitrile-based peptidomimetic inhibitors and test their inhibitory effects on the 3C-like proteases. The best inhibitor, Cbz-AVLQ-CN, was able to inhibit 3C-like proteases from a broad spectrum of coronaviruses (human CoV 229E, NL63, OC43, HKU1, SARS, and IBV) with IC_{50} values of 1.3 – 3.7 mM. Our work provides invaluable insights into the design of drugs to combat future outbreak of coronavirus infections.

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Molecular Epidemiological Investigation of PARV4-related Viruses in Hong Kong and Southern China

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Introduction: Many emerging infectious diseases are zoonoses that cause epidemics in humans after overcoming the inter-species barrier. Identifying possible animal origins or counterparts of emerging viruses is important in understanding their epidemiology, evolution and potential for re-emergence. Parvoviruses are widespread pathogens that cause a wide range of disease in animals. Human parvovirus 4 (PARV4) was identified in 2005, but the epidemiology, mode of transmission, clinical significance and origin of this newly identified virus are still poorly understood. In particular, unlike the other human parvoviruses with related viruses in animals, PARV4 was phylogenetically distinct without closely related animal counterparts.

Methods: To identify possible animal origins of PARV4 or related viruses, a surveillance study for PARV4-like viruses in human and animal samples was conducted. Nearly full-length sequences of the identified PARV4-related viruses from sheep, swine, cattle and human were determined and analyzed.

Results: PARV4-like viruses were detected by PCR among 44.4% (148/333) of porcine samples (including lymph nodes, liver, serum, nasopharyngeal and fecal samples), 13% (4/32) of bovine spleen samples, 1.8% (2/110) of bovine liver samples, 71.4% (10/14) of ovine spleen samples, 66.7% (6/9) of ovine liver samples and 2% (7/362) of human serum samples sent for HIV and HCV antibody tests. Four distinct parvoviruses were identified, including three novel parvoviruses, ovine hokovirus (OHoV), porcine hokovirus (PHoV) and bovine hokovirus (BHoV), and PARV4 from humans. Analysis of genome sequences from four OHoV, seven PHoV, five BHoV and one PARV4 strains showed that the animal parvoviruses were most similar to PARV4 and together formed a distinct cluster within Parvoviridae. The four parvoviruses also differed from other parvoviruses by their relatively large predicted VP1 protein and the presence of a small unique conserved putative protein. Based on these results, we propose a separate genus, Hokovirus, to describe these three parvoviruses. The co-detection of porcine reproductive and respiratory syndrome virus, the agent associated with the recent "high fever" disease outbreaks in pigs in China, from our porcine samples warrants further investigations.

Discussion and Conclusions: Three novel parvoviruses, OHoV, PHoV and BHoV, were discovered in Hong Kong. Animal viruses closely related to PARV-4 were identified in porcine and bovine samples, suggesting that interspecies transmission could have occurred during their evolution. This has dramatically improved the understanding of the origin of PARV-4 and this group of novel viruses. Since these animals are common food animals, further studies are warranted in studying the pathogenesis and epidemiology of these novel parvoviruses.

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Anti-ganglioside Antibodies were not Detected in Human Subjects or Mice Infected with or Vaccinated Against 2009 Pandemic Influenza A (H1N1) Virus

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Background: Mass vaccination against influenza A virus (H1N1) was terminated in USA in 1976 due to significantly increased risk of Guillain-Barré syndrome (GBS), an acute, immune-mediated demyelinating disorder that can cause paralysis and death. Anti-ganglioside antibodies underlying the development of GBS were reported by another group to be induced in mice receiving an H1N1 vaccine produced in 1976, another vaccine against influenza, or influenza hemaggulutin (HA).

Objectives: The overall goals of this pilot study are to verify the ability of swine-origin H1N1 influenza virus HA to induce anti-ganglioside antibodies in mice and if possible to map the peptide sequence associated with this induction. Because human subjects infected with or vaccinated against pandemic H1N1 2009 (pdm09) were available after the submission and approval of our proposal, we also expand our study to analyze anti-ganglioside antibodies in individuals infected with or vaccinated against pdm09, including eight confirmed cases of post-vaccination GBS.

Methods: PANFLU.1 vaccine against pdm09 in Freund complete adjuvant was used to immunize C57 mice. Serum samples from infected or vaccinated human subjects or mice were collected. Antibodies against GM1 and other gangliosides were detected by commercial ELISA kits. HAI titers in serum samples were also analyzed.