Advances in Biomedical Research

The Sixth Annual Joint Scientific Symposium of NIH/FDA CAA and Washington DC Chapter of SCBA

The National Institute of Health (NIH)/US Food and Drug Administration (FDA) and Chinese American Association (CAA) and the Washington DC Chapter of Society of Chinese Bioscientists in America (SCBA) have successfully sponsored five consecutive annual joint scientific symposia since 1994. Leading experts at the forefront of various biomedical fields have been invited to present their research findings. These joint symposia have sparked spirited discussions and led to productive collaborations among Chinese biologists in the great Washington DC metropolitan area.

The sixth symposium was held in the NIH Bethesda campus (Bldg. 10, Lippsett Amphitheater) on October 9, 1999. This year’s symposium highlighted recent advances in biomedical sciences, especially the HIV and tumor biology that has attracted attention from both the scientific community as well as the popular press. HIV infection has been a serious worldwide health concern. Prevention, intervention and treatment of this deadly disease represent major challenges and opportunities to biomedical researchers. Three talks were devoted to this issue. The first two presentations by Drs. Sylvia Lee-Huang (NYU) and Hao Chia Chen (NIH) described the exciting discovery of new anti-HIV agents of promising clinical potentials. Several new anti-HIV agents including (1) MAP30 and GAP31 from medical plants and (2) AVL and AVR from urine of pregnant women and (3) RNase U and urinary lysozyme C from pregnant women were described. The third talk by Dr. Kuan-Teh Jeang (NIH) focused on the pivotal role of CXCR4 in the treatment of HIV infection.

The EGF signaling pathway plays an important role in tumorigenesis and angiogenesis. A new regulatory protein CAIR-1 that can control the releasing PLC-g in response to growth factor stimulation was presented by Dr. Howard Doong (NIH). Defects in the mismatch repair genes have been identified in various tumors. The human MYH, a homologue of Escherichia coli MutY, have been identified. Dr. A-lien Lu (University of Maryland) presented the linkage between inactivation of hMYH and tumor progression.

Immunological regulation and response to diseases via an intricate network of pathways in the human body: three presentations addressed this topic; Dr. XiaoDong Li (Johns Hopkins University) presented the rapid identification of differentially expressed genes in human TH2 cells and their functional significance in allergic asthma. Dr. J. Qian (American Red Cross) focused on the prevention and treatment of hemophilic inhibitors by exploiting the CD40L/CD40 and B7/CD28 pathways to develop an anti-hemophilic inhibitor in the murine model. Dr. Tiang-Li Wang (Johns Hopkins University) described the use of nucleic acid vaccine to prevent human papillomavirus-induced cervical cancer. Linkage E7 antigen with targeting signal of the MCH II pathway of LAMP-1 significantly enhances the potency of DNA vaccine.

Dr. Yufan Shi, Dr. Andrew Chang, Dr. T.-C. Wu, and Dr. Yunbo Shi provided valuable advice in organizing this symposium. We would like to thank Dr. M.K. Jeang, Cardiovascular Center, University of Texas of Health Sciences Center at Houston, the Science Division of Taipei Economic and Cultural Representative Office in US, and generous support from a number of companies. We especially thank Dr. K.-T. Jeang, the former president of NIH/FDA CAA, for his insights and enthusiastic and continuing support for this joint symposium.

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In Search of Novel Anti-HIV Agents

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For the past several years, we have been searching for novel antiviral and anti-tumor agents from nature products. From hundreds of samples investigated, we identified, purified to homogeneity, characterized and cloned a new class of anti-HIV agents with high potency and low toxicity from distinct and unrelated sources. The first group consists of anti-HIV proteins MAP30 (Momordica Anti-HIV Protein 30 kD) and GAP31 (Gelonium Anti-HIV Protein 31 kD) from medicinal plants and the second group consists of AVL (anti-viral lysozyme) and AVR (anti-viral RNase) from urine of pregnant women. MAP30 and GAP31 are isolated from medicinal plants Momordica charantia and Gelonium multiflorum, also known as bitter melon and Himalayan fruit, respectively. These compounds are unique in that they not only inhibit de novo infection by HIV-1 but also block the replication of the virus in already infected cells. We found that they affect HIV-1-infected cells with EC50 (effective concentration at 50% inhibition) in the subnanomolar range (0.2–0.3 nM). They show no apparent cytotoxic or cytostatic effects on normal human cells even at 1,000-fold higher dose levels. MAP30 and GAP31 possess multiple therapeutic targets at different stages of the HIV-1 life cycle. We have characterized at least three biological activities that may be relevant to their therapeutic use. The first is an RNA N-glycosidase activity that cleaves the link between a ribose and adenine A4324 of 28S ribosomal RNA. This inactivates the 60S ribosomal subunit and inhibits polypeptide chain elongation. The second is a DNA topological inactivation activity that renders HIV-LTR topologically inactive as substrates for DNA gyrase. This topoinactivation is similar to the effect of cellular topoisomerases in the presence of topoisomerase inhibitors. The third is inhibition of each of the three reactions catalyzed by HIV-1 integrase: 3' processing of the viral DNA, strand transfer, and cleavage at the viral/target junction. It is thus important to define the extent to which each of these mechanisms contribute to desired antiviral and antitumor actions or to undesired cytotoxicity. We carried out structural and activity mapping of MAP30 and GAP31 by X-ray diffraction of crystals and by limited proteolysis. We identified and isolated proteolytic fragments of MAP30 and GAP31 that are fully active against HIV-1 but not in ribosome inactivation. These peptides are as active as their parent molecules in HIV-1 inhibition with EC50 in the range of 0.2–0.4 nM. They inhibit HIV-integrate activity and HIV-LTR topological interconversion, but they do not inhibit ribosome activity. These results demonstrate that the antiviral activity of MAP30 and GAP31 is independent from their ribosome-inactivating protein activity. This is of great significance and may provide useful insights in the design and development of antiviral and anti-tumor agents with specific therapeutic targets toward viral-infected and/or tumor cells, without cytotoxicity towards cellular targets. The second group of antiviral agents consists of AVL and AVR. To our knowledge, this is the first report that lysozymes and ribonucleases possess anti-HIV activity, and the first identification of these proteins as components present in crude β-core preparations that contribute to its anti-HIV effects. They represent a totally new class of therapeutic agents because they are naturally occurring human proteins that modulate viral infection. Details of these studies are presented in the following abstract.

Mother Knows Best: From Pregnancy and the Discovery of Anti-HIV Proteins

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The transmission of HIV-1 from mother to fetus is rare during the first trimester of pregnancy when the secretion of hCG is high in the placenta. Consequently, hCG preparations were considered to have a role in the inhibition of HIV-1 transmission. Indeed, many hCG and its β-subunit (hCGβ) in particular were found to contain the anti-HIV activity both in vivo and in vitro studies. However, there has been controversy about whether some biological activities of hCGβ preparations are due to the β-subunit itself, or to other proteins present in the preparations. To determine whether proteins other than hCGβ itself might contribute to the anti-HIV activity of hCGβ preparations, we fractionated commercial preparations derived from the urine of pregnant women. We found that a significant portion of the anti-HIV-1 activity is associated with the β-core fraction. The β-core is a dimer of two peptide fragments of hCGβ linked by disulfide bridges. When a β-core fraction was purified by reverse-phase HPLC, the pure β-core molecules identified by N-terminal amino acid sequencing and SDS-PAGE were completely devoid of any anti-HIV activity assayed by p24 production in chronically HIV-1-infected ACH2 lymphocytes and U1 monocytes. The bulk of anti-HIV activity was eluted behind the β-core fractions. Further purification of the fractions containing the anti-HIV activity by SDS-PAGE followed by Sephadex G-25 superfine, 18- and 18.5-kD fractions was identified by N-terminal amino acid sequencing as ribonuclease (RNase) U and 14 kD as urinary lysozyme C. Both purified enzymes exhibited not only respective authentic enzymatic activities but also anti-HIV activity. As expected, RNase U effectively degraded total RNA isolated from HIV-infected ACH2 lymphocytes. Similarly, ribonuclease A was found as 23 kD on SDS-PAGE in an extensively purified β-core preparation. We therefore designate the ribonuclease and lysozyme as anti-viral ribonucleases (AVR) and anti-viral lysozyme (AVL), respectively. Furthermore, commercially available lysozyme from chicken egg white, human milk, and human neutrophils and RNase A from bovine pancreas were demonstrated in these studies to possess activity against HIV-1. Since lysozyme is elevated in the urine of pregnant women and known to reduce the absorption of ectromelia virus, it may play important protective roles during pregnancy. This may explain why HIV infection from mother to fetus is rare. Collectively, these findings may offer new strategies for the treatment of HIV-1 infection.