Journal of Biomedical Science (2005) 12:565–569
DOI 10.1007/s11373-005-0655-x



# Biomedical Vignette

In the current issue:

### Nick-directed repair of palindromic loop mismatches in human cell extracts

Palindromic DNA sequences (inverted repeats) are common features of most genomes including that of human. Palindromic sequences of varying length play different roles in transcription regulation, chromatin structure and transposition [1]. However, this structure also poses special problems in DNA replication and repair, thus contributing to genome instability [2]. Due to its special structure, the central loop region of a palindrome is repaired differently from simple mismatches, which has been characterized in bacteria and human [3]. In this study the repair of these loops in human cells are further analyzed using an *in vitro* system, which suggests the involvement of a nick-directed repair pathway [4].

### Chromosomal organization, evolutionary relationship, and expression of zebrafish GnRH family members

Gonadotrophin-releasing hormone (GnRH) was released by hypophysiotrophic neurons to stimulate the release of gonadotrophins from the pituitary and acting as a key initiator of the hormonal cascade controlling the reproductive axis. However, current studies indicated that each vertebrate species expresses two or three GnRH forms in multiple tissues, and different GnRH exert pleiotropic actions via different classes of receptors [5, 6]. Kuo et al. isolated two cDNAs, gnrh3 and gnrh2, from zebrafish brain and closely examined genomic structure and expression pattern of these two genes during development [7]. The phylogenetic analysis and comparison of conserved syntenies in the region surrounding the *GnRH* genes from human, chicken, pufferfish, and zebrafish genomes have revealed interesting relationship of two zebrafish *gnrh* genes in evolution.

### Herpes simplex virus type 1 propagation in HeLa cells interrupted by *Nelumbo nucifera*

Infection of herpes simplex virus Type 1 (HSV-1) causes painful but benign manifestations and recurrent diseases, including oral herpes, herpetic keratitis, herpes encephalitis, etc. The recipients of organ transplantation and immunocompromised or cancer patients have a high risk for increased severity of HSV-1 infection [8]. Nucleoside analogue acyclovir is widely used for the systemic treatment of HSV-1 infection. However, the acyclovir-resistant HSV-1 infections have been found in immunocompromised patients such as transplant and AIDS patients. Kuo et al. reported that the active fraction NN-B-5 isolated from seeds of *N. Nucifera* was able to block HSV-1 multiplication without apparent cytotoxicity. Results suggest that the mechanism of antiviral action of NN-B-5 is mediated through the inhibition of expression of immediate early transcripts, such as ICP0 and ICP4 mRNAs, which, in turn, blocks the viral replication and progeny production [9].

### A FAK mutant that promotes cell invasion but inhibits tumor growth

Focal adhesion kinase (FAK) has been implicated to play a critical role in regulating integrin-mediated cellular functions including cell migration, cell cycle progression, and cell survival [10]. In this issue, Chang et al. [11] showed that the FAK Y397F mutant with a mutation at its major autophosphorylation site had differential effects on v-Src-stimulated cell transformation; it promoted v-Src-stimulated invasion, but on the other hand it inhibited the v-Src-stimulated anchorage-independent cell growth *in vitro* and tumor formation *in vivo*. These results suggest that the autophosphorylation site of FAK is dispensable for its ability to promote cell invasion; however, its mutation may allow FAK to gain a function for suppressing tumor growth.

### Possible role of triacylglycerol-rich lipoproteins in the down-regulation of adipose obese mRNA expression in rats re-fed a high-fat diet

Leptin, the product of obese gene (*ob*), is a hormone that is secreted from adipocytes. Leptin appears to play a major role in the control of body fat stores through coordinated regulation of feeding behavior, metabolism, autonomic nervous system and body energy balance in rodents, primates and humans [12]. Leptin released from fat cells is an important peripheral signal from fat stores, which modulates food intake. The expression of *ob* mRNA and secretion of leptin are low in fasting state, because leptin serves as an important signal from fat to the brain that the body is starving. In contrast, the expression of *ob* mRNA and secretion of leptin rapidly increase on re-feeding; the decrease in *ob* mRNA in the fasted rats is restored by insulin administration, indicating that insulin is an important regulator of the *ob* gene expression [13]. In this issue, Dr. Lu and his colleagues demonstrated for the first time that triglyceride(TG)-rich lipoproteins might act as another important regulator of *ob* gene expression [14]. They also showed that the levels of TG-rich lipoproteins were much higher in rats fed with high fat diet. These findings provide a good explanation that a weak action of dietary fat on satiation could induce a positive energy balance and lead to a gradual increase of body weight. It will be interesting and important to see whether the effect is also true in humans.

### Lipoprotein lipase gene and hypertension in Taiwan young hypertension study

Hypertriglyceridemia is associated with hypertension as a part of metabolic syndrome, an increasing threat to human health [15]. However, the mechanism behind it is poorly understood. A positive linkage signal between *LPL* and young-onset hypertension has been identified by Pan and colleagues as the strongest among 18 candidate genes [16]. In this report from the same group, Chen et al. [17] reported their fine mapping works with seven microsatellite markers flanking *LPL*, sequencing results for its promoter and exons, and an extended association study with the identified SNP. Using data from 213 people in 59 nuclear families of young-onset hypertension, *LPL* (GZ-14/GZ-15) marker in intron 6 of *LPL* gene was not only linked but also associated with hypertension. This result was confirmed in an independent case–control study with an intermediate phenotype of hypertension, i.e. hypertension with persistent elevated fasting triglyceride. They concluded that *LPL* variants may play a causal role in the development of hypertension in Taiwan Han Chinese.

### Cardiac cytochrome c oxidase and cardiac hypertrophy

Cytochrome c oxidase (COX) catalyzes the rate-limiting reaction of the mitochondrial oxidative phosphorylation (OXPHOS) and plays an essential role in maintaining normal cardiac function [18, 19]. However, the contribution of the mitochondrial components, the main source of energy for the cardiac hypertrophic growth induced by pressure overload, is not well understood. Experiments were performed in cardiac hypertrophy that was induced either by complete coarctation of abdominal aorta or by SHR. Results demonstrated a disproportionate increase in mitochondrial energy-producing cardiac hypertrophy [20]. In addition, the dysfunction of cardiac COX in SHR and complete coarcted rats, might be the key factor for the cardiac oxidative ability during cardiac hypertrophy. In conclusion, the present model provides a unique system to study the molecular basis underlying the adaptation of oxidative system in cells in response to the demand of hypertrophic growth.

### Proinflammatory role of substance P released by thoracic vagal efferent nerve stimulation

Thoracic vagus nerves (TVN) harbor tachykinin-containing sensory axons which carry sensory and pre-ganglionic parasympathetic fibers to the trachea, bronchial trees and lung [21]. Selective TVN efferent stimulation causes plasma extravasation on the ventral wall of the lower trachea and on the ipsilateral first- to fourth-order bronchi [22]. Li et al. [23] explored whether efferent TVN stimulation via substance P facilitates neurogenic inflammation via action of NF-κB activation and

reactive oxygen species (ROS) production in rats by using some specific inhibitors including $NK_1$ and $NK_2$ receptor antagonists and ROS scanvergers. They found that TVN efferent stimulation increases substance P release to trigger NF-κB-mediated ICAM-1 expression and ROS production in the respiratory tract.

### Thaliporphine and post-ischemia reperfusion Injury

The manifestations of reperfusion injury include arrhythmia, myocardial stunning, endothelial dysfunction and cell death [24]. Recently, it has been found that opioids can protect against post-ischemic myocardial infarction when given before reperfusion [25]. Thaliporphine is a phenolic aporphine alkaloid found in many medicinal plants such as *Lauraceae*. This compound has partial calcium ($Ca^{2+}$) channel-activating activity and strong sodium ($Na^+$) and potassium ($K^+$) channel-blocking activity, which may contribute to its antiarrhythmic action [26]. The aim of this study was to evaluate whether thaliporphine administered before coronary reperfusion could have the same cardioprotective effect as pretreatment before the ischemia-reperfusion period. Thaliporphine at 0.05 and 0.5 mg/kg were found to reduce the infarct size. Recovery of cardiac function was higher in the thaliporphine (0.5 mg/kg) group, as assessed by a significant improvement in the rats of pressure development ($+dp/dt_{max}$). This compound also reduced plasma creatine kinase and cardiac MPO activity. These protective effects afforded by thaliporphine were diminished by the opioid receptor antagonists (naloxone or naltrexone) and by the mitochondrial $K_{ATP}$ blocker 5HD. These results demonstrate that reperfusion therapy with thaliporphine protect cardiac injury through activation of opioid receptor and opening of mitochondrial $K_{ATP}$ channels, similar to morphine but with stronger activity.

### Myocardial dysfunction induced by food restriction is related to morphological damage in normotensive middle-aged rats

Food restriction (FR) has been shown to induce beneficial health effects. However, recent research suggests that FR may lead to left ventricular dysfunction and increase contraction and relaxation times in isolated papillary muscle of young rats. The mechanisms behind the changes in myocardial function with FR remain unknown. Sugizaki et al. [27] investigated the effects of chronic FR on cardiac and myocardial functions in middle-aged rats. Their data show that FR did not change left ventricular function, but increased time to peak tension and contraction duration, and decreased maximum rate of papillary muscle tension development. Ultrastructural alterations of myocardial cells were observed in most FR rats, suggesting that morphological damage may be related to intrinsic muscle performance depression.

### Activation of muscarinic $K^+$ channels by arecaidine propargyl ester in isolated guinea-pig atrial myocytes

Arecaidine propargyl ester (APE), a potent muscarinic acetylcholine agonist, was developed as a potential therapeutic agent for Alzheimer's disease [28]. However, APE has been shown to produce negative chronotropic and ionotropic effects in isolated atria [29]. In this communication, Chen [30] showed that APE activated muscarinic $K^+$ channels and increased potassium conductance in guinea-pig atrial myocytes. In addition, APE also shortened action potential duration in the same system. These findings are significant since they provide mechanism underlying the undesirable effects of APE in the cardiovascular system.

### Roles of the minor pseudopilins, XpsH, XpsI and XpsJ, in the formation of XpsG-containing pseudopilus in *Xanthomonas campestris pv. campestris*

During bacterial infection, delivering of hydrolytic enzymes and toxins by protein secretion is essential for bacterial pathogenicity. For Gram-negative bacteria, the transported proteins need to cross both inner and outer membrane through protein secretion machinery. In type II secretion pathway, at least 12 gene products are involved. Among them, five proteins are designated as pseudopilins due to their homology to type IV prepilin in sequence. The formation of a pilus-like pseudopilin structure has previously been suggested [31].

Using *Xanthomonas campestris pv. campestris* as a model, Hu et al. has previously reported that the major pseudopilin, XpsG, forms a pilus-like structure between cytoplasmic and outer membranes [32]. Studying the role of the minor pseudopilins in type II secretion pathway, Kuo et al. demonstrate that the major pseudopilin XpsG, and minor pseudopilins XpsH, -I and -J can form a multiprotein complex with a linearly ordered XpsG-XpsJ-XpsH-XpsJ interactive sequence [33].

## References


1. Haun R.S., Moss J. and Vaughan M., Characterization of the human ADP-ribosylation factor 3 promoter. Transcriptional regulation of a TATA-less promoter. J. Biol. Chem. 268: 8793–8800, 1993.
2. Tanaka H., Tapscott S.J., Trask B. and Yao M.-C., A conserved mechanism of palindrome formation and the initiation of gene amplification in mammalian cells. Proc. Natl. Acad. Sci. USA 99: 8772–8777, 2002.
3. Fang W.-H., Wang B.-J., Wang C.-H., Lee S.-J., Chang Y.-T., Chuang Y.-K. and Lee C.-N., DNA loop repair by *Escherichia coli* cell extracts. J. Biol. Chem. 278: 22446–22452, 2003.
4. Chuang Y.K., Cheng W.C., Goodman S.D., Chang Y.T., Kao J.T., Lee C.N., Tsai K.S. and Fang W.H., Nick-directed repair of palindromic loop mismatches in human cell extracts. J. Biomed. Sci. (this issue).
5. Lethimonier C., Madigou T., Munoz-Cueto J.A., Lareyre J.J. and Kah O., Evolutionary aspects of GnRHs, GnRH neuronal systems and GnRH receptors in teleost fish. Gen. Comp. Endocrinol. 135: 1–16, 2004.
6. Morgan K. and Millar R.P., Evolution of GnRH ligand precursors and GnRH receptors in protochordate and vertebrate species. Gen. Comp. Endocrinol. 139: 191–197, 2004.
7. Kuo M.W., Lou S.W., Postlethwait J. and Chung B.C., Chromosomal organization, evolutionary relationship, and expression of zebrafish GnRH family members. J. Biomed. Sci. (this issue).
8. Chibo D., Druce J., Sasadeusz J. and Birch C., Molecular analysis of clinical isolates of acyclovir resistant herpes simplex virus. Antivir. Res. 61: 83–91, 2004.
9. Kuo Y.C., Lin Y.L., Liu C.P. and Tsai W.J., Herpes simplex virus Type 1 propagation in HeLa cells interrupted by *Nelumbo nucifera*. J. Biomed. Sci. (this issue).
10. Parsons J.T., Focal adhesion kinase: the first ten years. J Cell Sci. 15: 1409–1416, 2003.
11. Chang L.-C., Huang C.-H., Cheng C.-H., Chen B.-H. and Chen H.-C., Differential effect of the focal adhesion kinase Y397F mutant on v-Src-stimulated cell invasion and tumor growth. J. Biomed. Sci. (this issue).
12. Zhang Y., Proenca R., Maffei M., Barone M., Leopold L. and Friedman J.M., Positional cloning of the mouse *ob* gene and its human homologue. Nature 372: 425–432, 1994.
13. Saladin R., De Vos P., Guerre-Millo M., Leturque A., Girard J., Staels B. and Auwerx J., Transient increase in obese gene expression after food intake or insulin administration. Nature 377: 527–529, 1995.
14. Chang M.L., Chen H.L. and Lu S.C., Possible role of triacylglycerol-rich lipoproteins in the down-regulation of adipose obese mRNA expression in rats re-fed a high-fat diet. J. Biomed. Sci. (this issue).
15. Eckel R.H., Grundy S.M. and Zimmet P.Z., The metabolic syndrome. Lancet 365: 1415–1428, 2005.
16. Pan W.H., Chen J.W., Fann C., Jou Y.S. and Wu S.Y., Linkage analysis with candidate genes: the Taiwan young-onset hypertension genetic study. Hum. Genet. 107: 210–215, 2000.
17. Chen P., Jou Y.S., Fann C.S.J., Chen J.W., Wu S.Y. and Pan W.H., Lipoprotein lipase gene is linked and associated with hypertension in Taiwan young-onset hypertension genetic study. J. Biomed. Sci. (this issue).
18. Tanji K. and Bonilla E.M., Neuropathologic aspects of cytochrome C oxidase deficiency. Brain Pathol. 10: 422–430, 2000.
19. Richter O.M. and Ludwig B., Cytochrome c oxidase-structure, function, and physiology of a redox-driven molecular machine. Rev. Physiol. Biochem. Pharmacol. 147: 47–74, 2003.
20. Kuo W.W., Chu C.Y., Lin J.A., Lin J.Y., Ying T.H., Hsieh Y.H., Chu C.H., Lin D.Y., Hsu H.H. and Huang C.Y., The profile of cardiac cytochrome c oxidase (COX) expression in an accelerated cardiac-hypertrophy model. J. Biomed. Sci. (this issue).
21. Belvisi M.G., Overview of the innervation of the lung. Curr. Opin. Phar. 2: 235–243, 2002.
22. Li P.C., Huang H.T. and Liang J.T., Neurophysiology effects of recurrent laryngeal and thoracic vagus nerves on mediating the neurigenic inflammation of the trachea, bronchi, and esophagus of rats. Auton. Neurosci: Basic Clin. 88: 142–150, 2001.
23. Li P.C., Li S.C., Lin Y.J., Liang J.T., Chien C.T. and Shaw C.F. Thoracic vagal efferent nerve stimulation evokes substance P-induced early airway bronchonstriction and late proinflammatory and oxidative in jury in the rat respiratory tract. J. Biomed. Sci. (this issue).
24. Hearse D.J. and Boli R., Reperfusion induced injury: manifestations, mechanisms, and clinical relevance. Cardiovas. Res. 26: 101–108, 1992.
25. Gross E.R., Hsu A.K. and Gross G.J., Opioid-induced cardioprotection occurs via glycogen synthase kinase beta inhibition during reperfusion in intact rat hearts. Circ. Res. 94: 960–966, 2004.
26. Chang W.L., Lee S.H. and Su M.J., Attenuation of post-ischemia reperfusion injury by thaliporphine and morphine in rat hearts. J. Biomed. Sci. (this issue).
27. Sugizaki M.M., Carvalho R.F., Aragon F., Padovani C.R., Okoshi K., Okoshi M.P., Zanati S.G. and Dal Pai-Silva M., Myocardial dysfunction induced by food restriction is related to morphological damage in normotensive middle-aged rats. J. Biomed. Sci. (this issue).
28. Moser U., Lambrecht G., Wagner M., Wess J. and Mutschler E., Structure–activity relationships of new analogues of arecaidine propargyl ester at muscarinic M1 and M2 receptor subtypes. Br. J. Pharmacol. 96: 319–324, 1989.
29. Barlow R.B. and Weston-Smith P., The relative potencies of some agonists at M2 muscarinic receptors in ileum, atria and bronchi. Br. J. Pharmacol. 85: 437–440, 1985.

30. Chen H., Activation of muscarinic K+ channels by arecaidine propargyl ester in isolated guinea-pig atrial myocytes. J. Biomed. Sci. (this issue).
31. Sauvonne N., Vignon G., Pugsley A.P. and Gounon P., Pilus formation and protein secretion by the same machinery in *Escherichia coli*. EMBO J. 19: 2221–2228, 2000.
32. Hu N.T., Leu W.M., Lee M.S., Chen A., Chen S.C., Songn Y.L. and Chen L.Y., XpsG, the major pseudopilin in *Xanthomonas campestris pv. campestris*, forms a pilus-like structure between cytoplasmic and outer membrane. Biochem. J. 365: 205–211, 2002.
33. Kuo W.W., Kuo H.W., Cheng C.C., Lai H.L. and Chen L.Y., Roles of the minor pseudopilins, XpsH, XpsI and XpsJ, in the formation of XpsG-containing pseudopilus in *Xanthomonas campestris pv. campestris*. J. Biomed. Sci. (this issue).