J Biomed Sci (2008) 15:669–673
DOI 10.1007/s11373-008-9279-2

BIOMEDICAL VIGNETTE

Biomedical vignette

Published online: 26 October 2008

Functional role of CTGF in cancer progression

Connective tissue growth factor (CTGF) was initially discovered in 1991 as a secreted protein in the conditioned media of cultured human umbilical vascular endothelial cells [1]. CTGF is a member of the CNN family of secreted, matrix-associated proteins encoded by immediate early genes that play various roles in angiogenesis and tumor growth [2]. Although papers and reviews on CTGF have been published, this review [3] focuses on the functional role of CTGF in cancer progression. The influence of CTGF expression on the behavior and progression of various cancer cells, as well as its regulation on various types of protein signals and their mechanisms are highlighted. Although CTGF expression seems to be associated with progression of many kinds of cancers, its expression may have tumor suppressive effects in a few cases such as lung adenocarcinoma cells, colorectal cancer cells and oral squamous carcinoma cells.

CGcgh: a tool for molecular karyotyping using DNA microarray-based comparative genomic hybridization (array-CGH)

To analyze the rare events of single-copy DNA aberrations, a MATLAB-based, array CGH analyzing program, Chang Gung comparative genomic hybridization (CGcgh) was employed to survey chromosomal amplifications and deletions in fetal aneuploidies or cancer tissues [4]. The analyzed chromosomal data are displayed in a graphic interface, and CGcgh allows users to launch a corresponding G-banding ideogram. In 15 karyotyped samples, the CGcgh program outperformed other programs and CGcgh supported the data generated from cDNA microarrays, spotted oligonucleotide microarray and Affymetrix Human Mapping Arrays.

A computational screen for C/D box snoRNAs in the human genomic region associated with Prader-Willi and Angelman syndromes

To identify snoRNAs (small nuclear RNAs) in the deletion of human chromosomal region 15q11-q13 related to Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS), computational scanning and screening and a novel hybridization energy test were used to identify all the snoRNAs. Three previously unknown methylation snoRNAs targeting ribosomal 18S and 28S RNAs and two snoRNAs targeting serotonin receptor 2cmRNA were identified [5]. The application of the present method to the PWS/AS region of human genome identified 11 snoRNAs, three of which pass the hybridization test. These snoRNAs require experimental confirmation. Present hybridization test can be incorporated and automated with motif scanning for genome-wide studies.

Interactions between M protein and other structural proteins of severe acute respiratory syndrome coronavirus

The study by Hsieh et al. [6] in the current issue focuses on the protein–protein interactions that regulate SARS coronavirus assembly. This was performed by co-localization studies in cells that express structural viral proteins either individually or combined. Changes in localization induced

by the presence of other virus components indicate direct protein–protein interactions. These experiments indicate that the SARS M protein plays a pivotal role in virus assembly, similar to findings with other coronaviruses (Vennema et al., reference 14 in the paper). A model is presented to explain how the M protein interacts with multiple other structural proteins (S, E and NC) to facilitate virion assembly.

Severe acute respiratory syndrome coronavirus nucleocapsid protein confers ability to efficiently produce virus-like particles when substituted for the human immunodeficiency virus nucleocapsid domain

The assembly of virus particles is a complex, but ill-understood process. Some viruses like the human immunodeficiency virus type 1 (HIV-1) require only a single protein for the assembly of virus-like particles (VLPs) (Gheysen, reference 14 in the paper). The situation is more complex for the SARS coronavirus, which requires at least two structural proteins for VLP formation. The current study by Wang et al. [7] asked whether the SARS nucleocapsid (N) protein and fragments thereof can functionally replace the nucleocapsid domain of the self-assembling HIV-1 Gag protein. Using this novel chimeric VLP approach, the authors were able to map more precisely the SARS N domain that facilitates self-association and VLP formation.

Iron dictates the virulence of *Pseudomonas aeruginosa* in urinary tract infections

Host factors, such as iron concentration at the site of infection, may influence the virulence of *Pseudomonas aeruginosa* and hence the outcome of infection. Here, Mittal et al. [8] reported that the depletion of iron in the growth medium led to increased adherence of *P. aeruginosa* to uroepithelial cells and decreased phagocytosis of the bacteria. Furthermore, *P. aeruginosa* growth in iron-depleted medium showed increased renal bacterial load and tissue pathology in the challenged mice. The current study may provide insight into the pathogenesis of *P. aeruginosa* and facilitate the development of a preventive approach against *P. aeruginosa*-induced urinary tract infections.

Glycine input induces the synaptic facilitation in salamander rod photoreceptors

In vertebrate retinas, glycinergic synapses regulate glutamate transmissions in both synaptic layers, the inner plexiform layer (IPL) and outer plexiform layer (OPL). The function of glycinergic synapses in the inner retina has been known to inhibit glutamate transmissions, shaping light-evoked response in ganglion cells [9, 10]. However, little is known about the function of glycinergic synapses in the outer retina. In this issue, Shen et al. [11] reported that glycine depolarized rods and activated voltage-gated Ca^{2+} channels in the neurons, resulting in facilitation of glutamate release in photoreceptors and increase of the spontaneous excitatory postsynaptic currents in Off-bipolar cells. Furthermore, these authors reported that inhibition of a Cl^- uptake transporter NKCC1 effectively eliminated glycine-evoked depolarization in rods suggesting that NKCC1 maintains a high Cl^- level in rods, which is responsible for glycine-induced depolarization. This finding is quite significant since it reveals a new function of glycine in retinal synaptic transmission.

Dose-finding study with nicotine as an anti-antiseizure agent in PTZ-induced seizure model in mice

Nicotine exerts agonistic effect on neuronal nicotinic acetylcholine receptors and is reported to enhance release of excitatory neurotransmitter glutamate into the synapses [12]. Pentylenetetrazole (PTZ), on the other hand, produces seizures in small rodents [13] and is believed to act by antagonizing the inhibitory GABAergic [14] neurotransmission in central nervous system. In this issue, Medhi et al. [15] reported that subthreshold dose of nicotine pretreatment significantly decreased the CD50 value for PTZ. Sodium valproate but not topiramate, significantly inhibited PTZ-induced seizure. Nonconvulsive dose of nicotine significantly antagonized the protective efficacy of sodium valproate against PTZ-induced seizures. These findings are quite significant since they bear clinical relevance particularly amongst epileptic smokers who may show failure of efficacy of antiseizure agents and present with breakthrough seizure attacks due to nicotine.

Benzyl alcohol inhibits *N*-methyl-D-aspartate receptor-mediated neurotoxicity and calcium accumulation in cultured rat cortical neurons

Benzyl alcohol has been used as a preservative in some small multiple-dose vials of bacteriostatic sodium chloride or water for injection. However, there is concern that excipients such as benzyl alcohol may cause adverse reactions to neurons in premature infants [16, 17]. In this issue, Takadera and Ohyashiki [18] reported that benzyl alcohol inhibited NMDA-induced cytotoxicity. Furthermore, these authors showed that the protective effect of

benzyl alcohol on NMDA-induced toxicity is due to its effect in reducing NMDA receptor-mediated calcium accumulation, indicating that benzyl alcohol inhibits NMDA receptor activity. This finding is significant since it shows potential beneficial effect of benzyl alcohol on mature neurons against glutamate-induced neurotoxicity although it may have adverse effect on immature neurons.

Bone regeneration by bone morphogenetic protein-2

Bone morphogenetic proteins (BMPs) are the most potent class of all osteoinductive proteins, and BMP-2 has already been clinically applied to accelerate bone regeneration in both fracture and spinal fusion [19]. Bone narrow-derived mesenchymal stem cells (BMMSCs) have been shown to be able to differentiate in vitro toward osteogenic lineages, when treated with established lineage-specific factor [20]. Kim et al. [21] studied if a combination of the undifferentiated BMMSCs and BMP-2 delivered via heparin-conjugated PLGA nanoparticles (HCPN) would extensively regenerate bone in vivo. In the in vivo testing, the undifferentiated BMMSCs with BMP-2 with BMP-2-loaded HCPN induced far more extensive formation, indicating the feasibility of entensive in vivo bone regeneration by transplantation of undifferentiated BMMSCs and BMP-2 delivery via HCPN.

tbx5 and cardiac myogenesis gene

Previous studies indicated that T-box genes play an essential role in cell specification and morphogenesis [22, 23]. In vertebrates, *tbx5* plays a critical role in cardiac and upper limb development. Expression of *tbx5* at the appropriate dose, time and position is important for normal cardiac development. Zebrafish appears to be a well-established model used in studying the development of vertebrates. The zebrafish is an ideal model and has been receiving attention as a human disease model, because the species fertilizes embryos externally, the fetus develops rapidly, and it manages to survive without cardiac function for days. Thus, zebrafish was used as the model organism to facilitate our investigation on the causal relationship between *tbx5* and cardiac myogenesis during cardiac looping. Results demonstrated that in zebrafish, injection of *tbx5* morpholino antisense RNA caused changes of heart conformation, defect of heart looping, pericardium effusion, dropsy of ventral position and decreased heart rate. In conclusion, this study showed deficiency of *tbx5* might perturb cardiac looping progress, as well as the formation of atrium and ventricle, possibly through down-regulating cardiacmyogenesis genes such as *amhc*, *vmhc* and *cmlc2* [24].

A novel protein involved in heart development in *Ambystoma mexicanum* is localized in endoplasmic reticulum

The discovery of the naturally occurring *cardiac nonfunction* (*c*) animal strain in *Ambystoma mexicanum* (axolotl) provides a valuable animal model to study cardiomyocyte differentiation. It was shown that this recessive mutation, in homozygous animals, causes incomplete differentiation of the embryonic heart at heartbeat initiation stages [25] due to a lack of organized myofibril formation in homozygous mutant hearts [26, 27]. In this issue, Jia et al. [28] reported cloning of a peptide cDNA (N1) from an anterior-endoderm-conditioned-medium RNA library. Furthermore, the authors have shown a dramatic decrease of expression of N1 mRNA in mutant (*c*/*c*) embryos, indicating that the N1 gene is involved in heart development. These findings are quite significant since revealing the underlying molecular mechanisms of heart development will be an important step in finding cures for heart diseases.

Immunohistochemical assessment of cyclic guanosine monophosphate (cGMP) and soluble guanylate cyclase (sGC) within the rostral ventrolateral medulla

The rostral ventrolateral medulla (RVLM) in the caudal medulla oblongata is a major site for generation of neurogenic vasomotor tone. Nitric oxide (NO) in the RVLM plays an important role in central cardiovascular regulation by regulating the sympathetic vasomotor outflow [29, 30], and cGMP-dependent NO signaling in the RVLM is impaired in hypertension [31]. To date, no studies have described the immunohistochemical localization of neurons capable of expressing cGMP within the RVLM. In this communication, Powers-Martin et al. [32] sought to identify the cellular targets for NO in the RVLM by visualizing anatomical relationship of cGMP with the tyrosine hydroxylase (TH) or phenylethanolamine N-methyltransferase (PNMT) cell group. Double label immunohistochemistry for cGMP-immunoreactivity (IR) and TH or PNMT neurons failed to reveal cGMP-IR neurons in the RVLM of either normotensive Wistar-Kyoto rats or the spontaneously hypertensive rats. In addition, soluble guanylate cyclase (sGC)-IR was found throughout neurons of the RVLM, but did not co-localize with PNMT- or TH-IR neurons. These results indicate that within the RVLM, cGMP is not detectable using immunohistochemistry in the basal state and this raises the hypothesis that functional network inputs, such as the sympathetic baroreflex pathway are required to drive a sGC/cGMP cascade in the RVLM.

In vivo GM-CSF promoter-based assay for drug screening

In drug discovery research, in vitro cell-based screening systems are well established as methods for evaluation of candidate lead compounds. For example, in vitro assays of NF-κB [33] and COX-2 [34], two examples of drug targets, are employed to develop therapeutic strategies to counter inflammation. Since the regulation of immuno-modifiers is highly dependent on three-dimensional microenvironments, an in vivo assay can more accurately evaluate the effects of drugs on the expression of key cytokines. Su et al. [35] devised an in vivo, transgenic, human cytokine (e.g. GM-CSF) gene promoter assay using defined epidermal skin cells as test tissue. Test compounds were topically applied to mouse skin before or after gene gun transfection, using a cytokine gene promoter-driven luciferase reporter. Croton oil, an inflammation inducer, induced six-folds transgenic GM-CSF promoter activity in skin epidermis, and this effect was drastically inhibited by the phytocompound shikonin. These results demonstrate that this in vivo transgenic promoter assay system is cytokine gene-specific, and highly responsive to pro-inflammatory stimuli.

Arecoline and the 30–100 kDa fraction of areca nut extract differentially regulate mTOR and respectively induce apoptosis and autophagy—a pilot study

In Taiwan, chewing of betel quit causes oral cancer, which is the top sixth killer of all types of cancer. Betel quit consists of areca nut (AN), lime and influorescene of Piper betel. It was demonstrated previously that extracts of AN (ANE), but not those of lime, inflorescence of Piper betel, induced rounding of cell morphology and nuclear shrinkage in several carcinoma cell lines. In the present study [36], the MW of active principle was found to be between 30–100 KDa, which induced nuclear shrinkage, clearance of the cytoplasm, cleavage of LC3-1Q and appearance of autophagic vacuoles and acid vesicles. On the other hand, arecoline (Are) triggered caspase-3 activation, peri-nuclear condensation and micronucleation. Furthermore, ANE 30–100 K but not Are, inhibited the phosphorylation of rapamycin (m-TOR)-Ser2448. It indicates that different constituents of AN induces apoptosis or autophage of an oral cancer cell line.

Dioxin detection by FRET technique

To the present, utilization of high-resolution gas chromatography with high-resolution mass spectrometry (HRGC/MS) is recognized as the most efficient way for determining dioxin compounds [37]. However, the procedures of this detection system are not easy to carry out. The fluorescence resonance energy transfer (FRET) technique can precisely evaluate interaction between two molecules in cells, and the use of cyan-fluorescent protein (CFP) as an energy donor and yellow-fluorescent protein (YFP) as an energy acceptor has been reported as the most suitable combination of FRET signal detection [38]. Lin et al. [39] therefore established a FRET-based dioxin-detection assay. Aryl hydrocarbon receptor (AHR) and AHR nuclear translocate (ARNT) fused-cyan fluorescent protein 9CFP) and-yellow fluorescent protein (YFP) constructs were transiently co-transfected into rat hepatoma cell line H4IIEC3 cells. No FRET signals were detected in AHR-CFP-and ARNT-YFP-transfected cells. However, dioxin treatment upregulated FRET signals in these transfected cells in a dose-dependent manner, indicating the potential of FRET technique in the detection of dioxin-like compounds.

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