Calcium efflux pump, PMCA2, in human breast tissue with lactational change and as a therapeutic target in breast cancer

Amelia A. Peters, Wei C. Lee, Eloise Dray, Chanel E. Smart, Lynne Reid, Leonard da Silva, Sunil R. Lakhani, Sarah J. Roberts-Thomson, Gregory R. Monteith

The University of Queensland, Woolloongabba, Australia

Abstract

Calcium pumps and channels modulate cell proliferation and apoptosis by regulating intracellular calcium (Ca2+). The plasma membrane Ca2+ ATPase isoform, PMCA2, is a calcium efflux mechanism that extrudes Ca2+ from the cytosol into the extracellular space. PMCA2 has a restricted expression, including expression in cochlear hair cells and cerebellar Purkinje cells. PMCA2 expression is increased in mouse mammary glands during lactation where it plays a major role in the excretion of Ca2+ into milk; however, PMCA2 expression has not been assessed in human breast tissue exhibiting lactational changes. Our previous studies have shown that PMCA2 mRNA levels are elevated in some breast cancer cell lines and that pan-PMCA antisense attenuates the proliferation of MCF-7 breast cancer cells. However, the consequences of silencing PMCA2 in breast cancer cells are still not well understood. Our study assessed PMCA2 expression in breast tissue exhibiting lactational change and in human malignant breast tissue samples. The role of PMCA2 in the proliferation of breast cancer cells was also evaluated. Immunohistochemistry using a rabbit anti-PMCA2 antibody showed membranous PMCA2 expression in the luminal epithelium of breast tissue exhibiting lactational change. PMCA2 expression was assessed in human breast tumor samples assembled into tissue microarrays. Nine of 96 breast tumours (9.4%) showed membranous PMCA2 staining. PMCA2 expression did not significantly correlate with the breast cancer pathological markers, estrogen, progesterone or HER2 receptor status. High-content imaging demonstrated that PMCA2 silencing in MDA-MB-231 breast cancer cells is associated with a reduction in cell number and an inhibition of the percentage of S-phase positive cells. The effect of PMCA2 silencing combined with various cytotoxics (cisplatin, doxorubicin or mitomycin C) on cell proliferation was assessed in MDA-MB-231 cells using a kinetic imaging system (IncuCyte). The results showed that PMCA2 silencing promotes the effects of some cytotoxics. These findings indicate that PMCA2 protein expression is elevated during human lactation and in some breast cancers. Inhibitors of PMCA2 may represent a novel therapeutic strategy for some breast cancers.

http://dx.doi.org/10.1016/j.nhtm.2014.11.041

Antibody-based targeting of TNF-ligands for cancer therapy

Edwin Bremer

University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Abstract

The tumor necrosis factor (TNF) ligand and cognate TNF receptor superfamily constitute an important immunoregulatory axis pivotal for the correct execution of immune responses. TNF ligand and receptor family members among others are involved in induction of cell death in malignant cells as well as in providing co-stimulatory signals that help mount effective anti-cancer immune responses. This diverse and important regulatory role in immunity has sparked great interest in the development of TNFL/TNFR-targeted cancer immunotherapeutics. Here, I will discuss our cancer immunotherapeutic drug discovery and development program using selected examples of the TNF-ligand superfamily.

http://dx.doi.org/10.1016/j.nhtm.2014.11.042

Effect of repeated passaging and cell density on proliferation and differentiation potential of cord blood unrestricted somatic stem cells

Zeinab Demerdash, Hanan El-Baz, Kesmat Maher, Salwa Hassan, Faten Salah, Marwa Hassan, Mohamed Elzallat, Mohammed El-Shafei, Tamer Taha

Theodor Bilharz Research Institute, Cairo, Egypt

Abstract

The ease of culture expansion of unrestricted somatic stem cells (USSCs) represents one of their primary advantages in clinical strategies. However, genetic alterations during culture expansion undoubtedly affect their therapeutic potential. Telomere shortening with aging is another factor that leads to aberrant stem cell functioning, interfering with potential therapeutic designs. This study evaluates the effect of cell density versus passaging number on the proliferation rate of cord blood (CB)-USSCs, reflected on the telomere length, pluripotent transcription factors expression, and differentiation potential. Methodology: CB-USSCs were cultured at seeding densities of 5000, 500, 50, 5 cells/cm2. Cells from different passages of each seeding density were subjected to pluripotency genes (Oct4, Sox2, Nanog, klf4, c-Myc) and PDGFRa gene expression analysis, measurement of absolute telomere length by real-time PCR, and induction of differentiation into osteogenic, adipogenic, and chondrogenic lineages. Proliferation rate was expressed as population doubling (PD) and cumulative PD (CPD). Results: USSCs from earlier passages (P7) cultured at 5000 cells/cm2 showed the highest telomere length with high expression of pluripotency, and proliferation genes which decreased gradually with passaging till reaching their lowest level at P11 Moreover, their PD at P7 was 3 and CPD (P7-P11) was 12.8. USSCs cultured at 5 cells/cm^2 showed PD 12.9 at P7, with a higher expression of gene that plays an important role in proliferation (PDGFRa) than that of 5000 cells/cm2 at P7. Differentiation potentiality of 5000 cells/ cm2 at P7 was high with loss of differentiation at P11, while differentiation potentiality of 5 cells/ cm2 at P7 was much lower than that of 5000 cells/ cm2 at same passage. Conclusion: Taken together, the above results suggest the use of USSCs at earlier passages of 5 cells/cm2 cultures if a high expansion rate of CB-USSCs is required in therapeutic strategies, while USSCs culture at 5000 cells/cm2 is the best protocol if the therapeutic target is induction of USSCs differentiation.

This work was financially supported by the Science and Technology Development Funds (STDF) , Grant No. 1410.

http://dx.doi.org/10.1016/j.nhtm.2014.11.043

Transcatheter Aortic Valve Implantation (TAVI), the evolution, the 2nd generation the directions & the criterias of the future generations

Ernö Remsey-Semmelweis

The University Clinics of Giessen & Marburg, Marburg, Germany

Abstract

Transcatheter valve intervention are more advanced and also successful evolution of conventional cardiac surgery. The first step in cardiac surgery was performed on the beating hearts in the case of digital or instrumental mitral commisurotomy. Although open heart surgery stays always the “Gold Standard” procedure, because it is crucial in understanding via direct

"Visus" the valve diseases. Transcatheter technologies take advantages from the open heart technique using catheter based instruments for elderly and high risk patients. Transcatheter mitral valve commisurotomy was the first surgical therapy converted to transcatheter one in the 1980. Today with a new art of transcatheter technology, with more clinical efficacy and safety is this "The Procedure of Choice" with a faster recovery and less peri-operative pain. But although it seems the TAVI procedure is more effective at elderly high risk or non-operable patients. The German TAVR registry shows that at low risk population, the observed mortality & morbidity is higher than that population by the EUROSCORE. The key and the crucial point at the introduction of a new clinical technology is the optimal "TRANSLATION" to the daily human practice. The new interventional technology has to be supported – after previous excellent results of animal and all clinical phase studies - by "Clinical Evidence**!!!**". In this technology imaging is the crucial factor in the selection with and in the screening process, to guide patients to the right size and art of device selection, as well as in playing a fundamental role during procedures to guide the implant safely and effectively. In the future the best imagination with a real touch will be probably holographic by an almost "REAL " 3D or 4D anatomical peri-operative representation.

http://dx.doi.org/10.1016/j.nhtm.2014.11.044

PTEN as a therapeutic target in motor neuron diseases (ALS/SMA)

Ke Ning

University of Sheffield, Sheffield, UK.

Abstract

The tumor suppressor protein Phosphatase and Tensin Homolog deleted on Chromosome 10 (PTEN) is a member of the protein tyrosine phosphatase family that can negatively regulate the serine/threonine kinase Akt to exert its tumour suppressor function. In addition to its normal functions such as neuronal migration and neuronal size control, PTEN protein is involved in pathological processes surrounding neuronal injury such as those associated with brain ischemia, neurological and mental disorders. It has been shown that modulation of the PTEN/mTOR pathway promotes axon regeneration in the adult CNS. We have previously shown that down-regulating the expression of PTEN protects against ischaemic neuronal death in vitro and in vivo (Ning et al. 2014). Recently, we showed that PTEN knockdown via siRNA increases motor neuron survival in Amyotrophic lateral sclerosis (ALS) (Kirby et al. 2011) in vitro and spinal muscular atrophy (SMA) in vivo (Ning et al. 2010, Little et al., unpublished). Our preliminary data show that the PTEN inhibitor, bpV, promotes cell survival in NSC34 G93A motor neuronal cell line. We have also showed that PTEN silencing increases cell survival in iPS-derived motor neurons from human fibroblasts (D-J Yang et al., 2014). Taken together, PTEN inhibition results in neuroprotective effects on motor neuron survival in vitro and in vivo. The outcome of our studies provide evidence that PTEN is potential therapeutic target for neuroprotection in ALS or SMA patients and other neurodegenerative disorders.

http://dx.doi.org/10.1016/j.nhtm.2014.11.045

Poster Presentations

SRM-based quantification of malignant biliary stenosis biomarkers in human bile

Annarita Farina, Annie Adrait, Jean-Marc Dumonceau, Myriam Delhaye, Jean-Louis Frossard, Yohann Couté

University of Geneva, Department of Human Protein Sciences, Geneva, Switzerland

Abstract

The differential diagnosis of biliary stenosis is a critical problem for gastroenterologists. An early identification of malignant lesions would enable the rapid resort to surgical resection which currently represents the only potentially curative option. Unfortunately, the diagnostic value of all available methods (e.g. imaging technics, standard serum biomarkers) is limited by relatively poor accuracy and negative predictive value. Recently, our group and others highlighted new potential cancer biomarkers in bile by using comparative proteomic analysis. Nevertheless, to date, only a few candidates have been verified for their diagnostic performances in discriminating between malignant and non-malignant stenoses. In addition, no data have yet been collected on the simultaneous measurement of these proteins with the intent of evaluating the diagnostic interest of a panel of biomarkers. To overcome the limitation of classical verification tools and give a new impetus to the translation of bile biomarkers into clinical diagnostics, mass spectrometry-based quantification could represent a rapid and cost-effective opportunity thanks to its capacity for multiplexed, high-throughput analysis, combined with its analytical specificity and reliable quantification. Here we developed the first Selected Reaction Monitoring (SRM) assay for the multiplexed measurement of cancer biomarkers in human bile. For this purpose, 8 potential biomarker candidates previously highlighted by proteomic analysis were selected. Equal volumes of bile collected from patients presenting with malignant and non-malignant biliary stenosis were stacked on the top of a SDS-PAGE gel. Proteins were then digested in-gel with trypsin and proteotypic peptides of each candidate biomarker were quantified by nanoLC-SRM on a 5500-QTrap mass spectrometer (ABSciex) using heavy synthetic peptides as standards (PEPotecTM, Thermofisher). SRM data were finally analysed using Skyline software and manual validation. The developed assay proved to be valuable and reliable to quantify all the selected candidates. Moreover, the results confirmed the simultaneous overexpression of some of the proteins in bile samples from malignant stenoses. Overall, our data demonstrate the ability of SRM to quantify cancer biomarkers in human bile and emphasize the interest of using multiplexed SRM assays to assess the diagnostic potential of a panel of bile biomarkers in differentiating biliary stenoses. Work supported by the PRIME-XS consortium.

http://dx.doi.org/10.1016/j.nhtm.2014.11.046

Hemopexin, a potential biomarker for the diagnosis of chronic predisposition to acute kidney injury

Ana Isabel Morales Martín, Laura Vicente-Vicente, Marta Prieto, Alfredo G. Casanova Paso, Francisco J. Lopez-Hernandez, José Miguel López-Novoa

IBSAL-IECSCYL, University of Salamanca, Salamanca, Spain

Abstract

In the last years, the new concept of predisposition to acquire acute kidney injury (AKI) is emerging. This concept was observed in our group when experimental animals exposed to an absolutely subnephrotoxic acute treatment with certain drugs (e.g. gentamicin and cisplatin) developed AKI when they were treated with a second insult with another drug, while control animals exposed to the same second drug experimented no toxicity. On these grounds, we decide to study if chronic exposure to nephrotoxicants might induce this predisposition to AKI and investigate how to detect this condition by the search of predisposition biomarkers. To this end, rats (Sprague-Dawley) were treated with a subtoxic dosage of the experimental nephrotoxin uranyl nitrate (UN) in the drinking water for 22 weeks, or plain water (as control). After 21 weeks both groups were treated with subtoxic regime of getamicin during 7 days. Renal function was monitored by means of serum creatinine, serum urea, proteinuria, N-acetyl-beta-D-glucosaminidase and lactate dehydrogenase excretion measurement. After and before