

technologies, as well as a boom in fluorophore engineering towards advanced functionalities. This talk will review selected new approaches, such as non-invasive imaging techniques, light-sheet microscopy and the use of optical highlighters, to underscore their applicative potential for basic and clinical research.

<http://dx.doi.org/10.1016/j.nhtm.2014.11.004>

New biomarker-based strategies for a preventive and personalized diagnosis of acute kidney injury

Francisco J. López-Hernández

IBSAL-IECSCYL, University of Salamanca, Salamanca, Spain

Abstract

Acute kidney injury (AKI) is a very relevant and increasing health and socioeconomic problem worldwide. In critically ill patients, mortality to AKI may reach up to 50–80% of the cases. Even mild, spontaneously reversible episodes of AKI have a significant impact on medium and long term morbidity and mortality. Drug nephrotoxicity is among the most important causes of AKI, with 25% of the 100 most used drugs in intensive care units being nephrotoxic. AKI has been traditionally diagnosed when extensive renal damage gave way to signs derived from renal dysfunction, such as the elevation of plasma creatinine concentration, according to well established methods of stratification, including the AKIN and RIFLE criteria. In the last decade, further advance was provided by the identification of earlier markers of kidney injury, including KIM-1 and NGAL. Yet, more sensitive and specific markers, or combinations of markers, are needed to improve AKI diagnosis. However, new facets of AKI diagnosis emerge for a more personalized and preventive handling of this disease. In the last years, our group has been working on two new diagnostic concepts, namely: i) pre-emptive detection of drug-induced predisposition to AKI, as differentiated from early diagnosis; and ii) aetiological (drug-specific) diagnosis of AKI, potentially applicable to polymedicated patients.

Drug-induced predisposition to acute renal failure (ARF) is a facet of nephrotoxicity hitherto mostly uncharacterized, quite underestimated, and impossible to diagnose, which potentially has a high human and socioeconomic impact. Our study has identified urinary GM2AP as the first of a new class of biomarkers of the enhanced risk of suffering an acute renal failure after a subnephrotoxic treatment with gentamicin. Gentamicin-predisposed animals with no sign of renal injury develop ARF when exposed to a second potentially nephrotoxic drug, also given at subnephrotoxic doses that are harmless to non-predisposed individuals. Subnephrotoxic gentamicin did not alter renal GM2AP gene expression or protein levels, determined by RT-PCR and Western blot and immunostaining, respectively, nor was its serum level modified. Further experiments indicate that, likely, the origin of the increased level of GM2AP in the urine might be a defective tubular handling of this protein as a consequence of gentamicin action. Markers of risk may revolutionize the prevention of ARF by enhancing our monitoring capacity of acquired predisposition to ARF, in a pre-emptive manner. With regard to the aetiological diagnosis of drug nephrotoxicity, we have identified regenerating islet-derived protein III beta (reg IIIb) and gelsolin as potentially differential urinary markers of gentamicin's nephrotoxicity. Indeed, both reg IIIb and gelsolin urinary levels differentiate the nephrotoxicity caused by gentamicin from that caused by cisplatin. Reg IIIb is over-expressed in the kidneys of gentamicin-treated rats and poured into the urine, whereas gelsolin proceeds from the glomerular ultrafiltrate. Our results pose a proof-of-concept for the aetiological diagnosis of AKI through the biochemical analysis of the urine, with potential application for an enhanced drug theranostic and a more personalized medicine of polymedicated and critically ill patients at multifactorial risk of AKI. Furthermore, our studies have identified new urinary markers that differentiate ischemic from toxic acute kidney injury.

<http://dx.doi.org/10.1016/j.nhtm.2014.11.005>

Targeting microRNAs: Towards a new tailored therapy for hepatocellular carcinoma

Raffaele Baffa

Sanofi, Cambridge, MA, USA

Abstract

Hepatocellular carcinoma (HCC) remains a significant unmet medical need with very limited therapeutic options available. Although microRNA-21 (miR-21) has been shown to be upregulated in HCC, its contribution as an onco-miR to the maintenance of tumorigenic phenotype in liver cancer remains poorly understood. We have developed potent and specific single-stranded oligonucleotide inhibitors of miR-21 (anti-miR-21) and used them to interrogate dependency on miR-21 in a panel of 20 commercially available HCC cell lines in vitro. Upon lipid-mediated transfection, anti-miR-21, but not its mismatched (MM) control, caused significant de-repression of known direct targets of miR-21, inhibited survival of a large subset of HCC cell lines. Sensitive HCC cell lines showed dose- and time-dependent induction of caspase 3/7 activity upon treatment with anti-miR-21. In contrast, non-responder HCC cell lines failed to significantly upregulate caspase activity and maintained viability in the presence of anti-miR compound. To better understand the consequences of miR-21 suppression in HCC, we carried out global gene expression profiling of anti-miR-21 treated sensitive liver cancer cells. Striking enrichment in miR-21 targets was noted among upregulated transcripts. Key cellular processes affected by miR-21 inhibition, including deregulation of metabolic pathways, were identified by gene ontology analysis. In summary, our data suggest that inhibition of miR-21 merits further investigation in the treatment of hepatocellular carcinoma.

<http://dx.doi.org/10.1016/j.nhtm.2014.11.006>

RLIP76 protein reduces 4-HNE generated during oxidative stress and results in protection in well characterized animal models of acute radiation syndrome

Elizabeth K. Leffel, Casey Cunningham, Brian R. Sloat, Henry Hebel, Curt Bilby

Leffel Consulting Group, LLC, Berryville, VA, USA

Abstract

Background: Ionizing radiation induces lipid peroxidation and forms reactive oxygen species (ROS) within the cell. Due to their highly reactive state, ROS have short diffusion distances and quickly transfer unbalanced electrons to neighboring molecules. This transfer cycle continues until it finally culminates in the generation of reactive alkenals such as 4-hydroxynonenal (4HNE). Under normal conditions, intracellular 4HNE levels are controlled by conjugation to glutathione and are actively transported from the cell; RLIP76 protein has been determined to be the major transport protein involved in the efflux of 4HNE-conjugates. Radiation induces significant oxidative stress, and the increased levels of 4HNE conjugates overwhelm transport capacity. When this occurs, further conjugation is inhibited and free 4HNE levels rise, triggering apoptosis. It was hypothesized that adding exogenous RLIP76 protein would reduce 4HNE levels and correspondingly increase the recovery from acute radiation syndrome (ARS) or completely protect individuals exposed to lethal doses of radiation.

Methods: The National Institutes of Health has developed animal models of ARS so that medical countermeasures can be tested in accordance with the FDA Animal Rule, since testing in people is clearly not ethical. One of these models, the C57BL/6 mouse, has been successfully utilized by Terapio to evaluate the efficacy of recombinant human RLIP76 encapsulated in liposomes (RLIP76-PL). These studies were 30-day survival studies of mice exposed to total body irradiation of 7.45–8.75 Gy.

Administration of RLIP76-PL was subcutaneous or intramuscular on a prophylactic (administered up to 20 h prior to irradiation) or therapeutic (administered up to 36 h after irradiation) schedule.

Results: As a prophylactic, there was 100% survival compared to 33% of the controls when mice were given three doses and exposed to 8.1 Gy. As a therapeutic, the drug is not administered until 24hrs after irradiation and remarkably, there is 92% survival compared to 8% of the controls.

Conclusions: Combined data of many studies show that when compared to controls, treated mice exposed to LD50s, LD70s, or LD90s resulted in survival ranging from 50–80% over the controls. RLIP76-PL is a strong candidate to protect the population from acute radiation exposure.

<http://dx.doi.org/10.1016/j.nhtm.2014.11.007>

Cancer prevention strategies in different countries: Qualitative and quantitative differences

Antonio Facchiano

Istituto Dermopatico dell'Immacolata (IDI-IRCCS), Rome, Italy

Abstract

In this presentation quantitative as well as qualitative analyses are reported regarding the communication strategies in the field of cancer prevention in the English-speaking world as compared to Italian-speaking world. The quantitative analyses were based on the occurrence of specific keywords in the global literature accessible via internet as well as on the chromatic-complexity investigated in a total of 1400 images. Such images refer to the “prevention” field and were taken from internet English-domains and Italian-domains. This study represents a preliminary report regarding a novel linguistic approach to assess the efficacy of cancer prevention strategies. The conclusions indicate significant and relevant quantitative differences between communication strategies having language differences.

<http://dx.doi.org/10.1016/j.nhtm.2014.11.008>

Methodologies and limitations in the analysis of potential neuroprotective compounds derived from natural products

John Weber

Memorial University of Newfoundland, St. John's, Canada

Abstract

Polyphenols, such as flavonoids found in a variety of plant species, have attracted the attention of scientists, the public, and the media due to their potential use as nutraceutical products. The high quantities of polyphenols found in some berry species, e.g. *Vaccinium* species such as blueberries and lingonberries, and their reported antioxidant and anti-inflammatory properties, could be beneficial for brain aging and neurodegenerative disorders. The neuroprotective potential of various polyphenolic compounds have been validated using a variety of *in vivo* and *in vitro* techniques, and they are often evaluated initially using *in vitro* cell culture techniques in order to establish toxicity and effective concentrations. Both *in vivo* and *in vitro* methodologies have their respective advantages and disadvantages, including, but not limited to, cost, time, use of resources and technical limitations. This presentation is meant to elaborate on the inherent benefits and drawbacks of *in vitro* and *in vivo* methods for assessing neuroprotection, especially in light of proper evaluation of compound efficacy and neural bioavailability. For example, *in vivo* studies can better evaluate the effects of protective compounds and/or their metabolites on various tissues, including the brain, in the whole animal, whereas *in vitro* studies can better discern the cellular and/or mechanistic effects of compounds. In particular, I aim to address the question

of appropriate and accurate extrapolation of findings from *in vitro* experiments where compounds are often directly applied to cellular extracts, potentially at higher concentrations than would ever cross the blood-brain barrier to the more complex scenario of neuroprotection due to pharmacodynamics *in vivo*.

<http://dx.doi.org/10.1016/j.nhtm.2014.11.009>

A newly human developed diabody against CD99 delivers a lethal signal through p53 pathway reactivation in Ewing sarcoma cells and synergistically acts with doxorubicin

Katia Scotlandi, Clara Guerzoni, Valentina Fiori, Cristina Manara, Michela Pasello, Diego Moricoli, Mara Gellini, Maurizio Cianfriglia, Mauro Magnani

Rizzoli Institute, Bologna, Italy

Abstract

Ewing sarcoma is the second most frequent primary tumor of bone, preferentially occurring in children and adolescents. Despite significant improvements have been achieved in localized tumors thanks to dose intensification of chemotherapy, outcome of patients with metastasis at diagnosis remains grim. In addition, even in the most favorable situations, patients must face with important side effects which significantly impact their quality of life. Either scientific and patient communities are now very sensitive on the need of new drugs, which may reduce chemotherapy toxicity while maintaining effectiveness of current regimens. We present here a new engineered human bivalent single chain fragment variable diabody (C7 scFv diabody) directed against CD99, a transmembrane protein whose high expression characterizes Ewing sarcoma. The triggering of CD99 with C7scFv diabody induces rapid and massive Ewing sarcoma cell death through MDM2 ubiquitination and p53 reactivation. Accordingly, the most CD99-responsive Ewing sarcoma cells have transcriptional active p53 and greatly benefit from MDM2 degradation. CD99 triggering also potentiates the cytotoxic effect of doxorubicin *in vitro* and *in vivo* and reactivates p53 to a much greater degree, which in turn markedly increased expression of pro-apoptosis genes. Evaluation of Ki-67 labelling and apoptosis rate by TUNEL confirms the efficacy of the treatment in xenografts. In contrast, mesenchymal stem cells, though expressing high levels of CD99, show no p53 activation and escape death induced by CD99 C7scFv diabody. No sign of toxicity was observed in mice treated with anti-CD99 scFV C7 diabody. Overall, our data provide the rationale for the use of this newly developed anti-CD99 diabody in the treatment of patients with Ewing sarcoma.

<http://dx.doi.org/10.1016/j.nhtm.2014.11.010>

A big data platform to enable integration of high quality clinical data and next generation sequencing data

Joel Haspel

Oracle Health Sciences, London, UK

Abstract

Today, personalized medicine is closer to reality than ever before through targeted treatment, however, the substantial increase in data correspondingly requires scalable systems to continue to effectively manage the data and to remain current with advancing technology. As organizations move to advance translational research to achieve personalized medicine, researchers and clinicians must manage informatics, however, there is a shortage of fully