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

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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 modifed. 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 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.

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Targeting microRNAs: Towards a new tailored therapy for hepatocellular carcinoma

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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.

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

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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 conjugate 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.

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