DNA-methylation and autoantibodies based cancer diagnosis from body fluids

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Abstract

Special focus and aim of our research activities at AIT, the Austrian Institute of Technology, is to define reliable biomarkers suitable for early and non-invasive disease diagnosis from body fluids such as serum/plasma and saliva. Along a selection of research projects, which are described in more detail underneath, we will present and introduce the broad portfolio of high throughput technologies we successfully apply for diagnostic biomarker discovery and validation. As a first show case of successful non-invasive disease biomarker discovery we will present a study where we investigated and compared the genome wide methylation patterns of lung cancer patients, suffering from lung fibrosis, patients with COPD (chronic obstructive pulmonary disease), and DNA samples derived from healthy lungs. Along this study we could identify specific methylation patterns for each of these lung diseases. After quantitative PCR validation of 240 disease specific methylation markers in the discovery sample set, the 90 top markers were picked and applied for serum testing (n=204). When we applied gradient boosting classification for differential diagnosis of tested lung diseases and healthy controls an AUC value of 0.95 was reached here to separate cancer from all other non-cancer samples whereas in differential diagnosis of healthy-, COPD and fibrosis patients AUC values of 0.71 and 0.49 were obtained for fibrosis, respectively COPD. Thus in case of COPD the presented method may be used to monitor cancer risk within COPD patients. Our second show case comprises a study where we screened cancer patients’ sera for tumor-specific antibody profiles using an in-house developed 16k protein-microarray. This methodology, which will be described in detail, enabled us to define different tumor-associated antigen (TAA) classifier panels for the big 4 cancer entities (breast, colon, prostate and lung cancer) which all showed very promising classification successes in distinction of patients versus controls. We will further present preliminary data obtained when comparing serum and saliva autoantibody profiles of breast-cancer patients and healthy controls.

Mass spectrometry-based quantification of malignant biliary stenosis biomarkers in human bile

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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.

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The changing face of epidemiology of systemic fungal infections

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Abstract

Invasive fungal diseases (IFDs) are an increasingly common complication in critically ill patients in Europe and are frequently fatal. Because of changes in treatment strategies and the increased use of antifungal prophylaxis, the epidemiology of IFDs has changed substantially in recent years and infections due to Candida species are no longer the majority in many institutions. In contrast, the emergence of non-Candida IFDs such as aspergillosis, urcmycosis and fusariosis has increased. Rates of IFD-related mortality in Europe depend on the pathogen, geographical location and underlying patient characteristics, with rates ranging from 26 to 50% for Candida infections and from 38 to 80% for invasive aspergillosis. Early initiation of antifungal therapy is critical for improving outcomes; however, this is complicated by the difficulty in diagnosing IFDs rapidly and accurately. Choice between agents should be based on a variety of factors, including spectrum of activity, adverse events, drug interactions, route of administration, clinical efficacy of individual agents and local epidemiology.

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Anti-tumor effects of the human monoclonal antinuclear antibody on the HEp-2 cells

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Abstract

Function of autoantibodies from patients with autoimmune diseases in malignancies development is not clear yet. It has been reported that a cell-penetrating lupus autoantibody, 3E10, which was isolated from a mouse model of systemic lupus erythematosus (SLE), has been a potential targeted therapy for DNA-repair deficient malignancies. We have got four human monoclonal antinuclear antibodies from patients with autoimmune disease, 3B5, 3C1, 3E8 and 4F3. Our data showed that four antibodies could combine HEp-2 cells and display different nuclear types as antinuclear antibody (ANA). Also, these four ANAs can inhibit HEp-2 cells proliferation. We think these antibodies may be potential antibody drugs to cancer therapy. However, the function and mechanism are not clear. Further study, we want to clarify the effects of four ANAs on proliferation of various cancers cells and to investigate the mechanism of four ANAs affecting various cancers cells proliferation and their targets. This may be a new mechanism of malignancies development in patients with autoimmune diseases, and provide novel angle of autoantibody function study.

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Rapid saliva test for varicella zoster virus

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Abstract

Varicella zoster virus (VZV) is a ubiquitous human herpesvirus typically causing childhood varicella (chickenpox) at which time a life-long latent infection is established in ganglionic neurons throughout the neuraxis. Reactivation of latent virus, typically in the elderly and immunocompetent usually causes zoster (shingles) but can also result in serious neurologic disease. In cases of vasculopathy, meningencephalitis and myelitis where VZV is suspected, diagnosis requires detection of virus DNA or antibody in CSF. In collaboration with NASA, VZV DNA was found in saliva of health astronauts suggesting asymptomatic virus reaction due to the stress of spaceflight. This lead to a series of studies indicating virus DNA can be found in saliva of patients with VZV associated neurologic disease. With the goal of eliminating the need for lumbar puncture to diagnose VZV associated neurologic disease; we developed a rapid saliva test for the detection of VZV DNA in saliva that can be used in space as well as on Earth. Herein the test and its potential application will be present.

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Novel approaches for the supportive extracorporeal therapy of sepsis: Towards personalized treatment

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Abstract

Sepsis and sepsis-associated multiple organ failure are associated with extensive tissue damage caused by over-activation of the innate immune system and by the excessive release of inflammatory mediators. The development of targeted therapies for sepsis remains a major challenge due to the complex network of inflammatory mediators involved in the septic process. Early detection and timely therapeutic intervention are crucial for improved outcome of patients with sepsis. Currently however, the diagnosis of sepsis and its severity relies on clinical criteria only. The heterogeneity of septic patients, the application of supportive extracorporeal therapies to modulate the concentration of inflammatory mediators requires diagnostic tools to monitor the inflammatory profile of the patients in order to identify the optimal time window for application of supportive therapies. Here, we report on the development of extracorporeal adsorption systems for cytokine modulation and on the development and validation of a novel array technology to detect markers of inflammation (interleukins 6 and 10,