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Metabolomics: Perspectives on potential biomarkers in organ transplantation and immunosuppressant toxicity

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ABSTRACT

Organ transplantation is the treatment of choice for many end stage diseases. The development and appropriate use of new immunosupressants have considerably improved the outcome of patients in the last decades. However, noninvasive, sensitive and specific biomarkers for early detection of complications leading to graft dysfunction are still needed. Current transplantation monitoring mostly relies on non-specific biochemical tests whereas diagnosis of rejection is generally based on invasive procedures such as biopsies. New approaches based on large scale profiling of body fluids and tissues are needed to address the complexity and multifactorial aspect of organ transplantation complications. Metabolomics aim to characterize and quantify the metabolome, which is the collection of the low-molecular weight compounds rising from metabolic pathways. Extracted from tissues or detected in body fluids, the small molecules are measured using nuclear magnetic resonance spectroscopy or mass spectrometry. By profiling the downstream products of cellular activity, metabolomics is most likely to represent the immediate cellular response to stresses. Diagnostic applications have been proposed in cancer, cardiovascular diseases, kidney diseases, neurological diseases and many more. This review will focus on the potential applications of metabolomics in organ transplantation including follow up of graft function recovery, diagnostic of alloimmune rejection as well as monitoring of immunosuppressant toxicity.

1. Introduction

Our better understanding of the involvement of immune system leading to the development and appropriate use of immunosuppressant drugs in organ transplantation have considerably improved the outcome of patients in the last decades [1]. However, noninvasive, sensitive and specific biomarkers for early detection of the associated complications leading to graft dysfunction and rejection are still needed [2,3]. Currently, standard follow-up is generally limited to non-specific tests such as serum creatinine measurements for kidney transplants, whereas the gold standard for diagnosis is the histopathology analysis of organ biopsies [4]. Tissue sampling requires invasive procedures and the diagnosis potential is sometimes limited by the heterogeneity of the underlying histological processes [2,5]. Integrative systems biology strategies are therefore increasingly applied to decipher the changes associated with complex multifactorial and chronic diseases such as kidney diseases [6,7], cancers [8–11], cardiovascular diseases [12–14], and neurological diseases among others [15,16]. Similarly to genomics, transcriptomics and proteomics, metabolomics is a large scale profiling strategy that allows the identification of new biomarkers [2,17]. It is defined by the high-throughput quantitative measurement of low-molecular weight compounds, generally under 1500 Da [18–21]. It investigates the metabolome, which is the collection of all the metabolites found in biofluids and tissues of a biological system. Based on the current Human Metabolome Database (HMDB), there are about 40 000 metabolites identified in human, whereas around 10,000 were identified in biofluids [22].

Metabolomics typically relies on nuclear magnetic resonance (NMR) and mass spectrometry (MS) methods that support the detection and quantification of hundreds to thousands metabolites in a single experiment (Table 1; [23]). The main application of NMR spectroscopy in metabolomics resides in its capacity to detect and quantify the abundance of molecules containing ^{1}H and ^{31}P isotopes in natural conditions and ^{13}C and ^{15}N isotopes in isotope-enriched conditions [24,25]. NMR is particularly well suited for metabolomics studies since it is a robust, highly reproducible and non-destructive high-throughput method that allows metabolites identification and quantification [24,26]. On the other hand, MS is a widespread analytical tool that is increasingly used in clinical biochemistry laboratories. It involves the ionization of metabolites present in samples and separation based on the mass/charge ratio (m/z) [27]. The high specificity and sensitivity of this approach allows the identification and quantification of thousands of metabolites, requiring only a small amount of sample and makes it a

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Table 1
Advantages and limitations of NMR- and MS-based metabolomics.

	NMR	MS
Experimental aspects[a]		
Settings for biofluid sample	1D ^{1}H and 1D ^{31}P	GC/LC/CE-MS
	2D CPMG	ESI-Q-TOF
	2D HSQC	ESI-Q-orbitrap
	2D COSY	MALDI-TOF
	2D TOCSY	
Settings for tissue sample	HRMAS	MALDI-TOF
Settings for in vivo studies	Possible with MRS	No
Average metabolites detection	< 200	500 +
Equipment		
Equipment cost	Very high	Moderate to high
Equipment maintenance cost	High	Moderate
Data analysis automation	Yes	Yes
Experimental time	5 min for 1D	Up to 60 min when combined with chromatography
	Up to 60 min for 2D	
General advantages	Non-destructive	Widespread
	Intrinsically quantitative	High sensitivity (pg-ng)
	Minimal sample preparation	High resolution
	Robust and reproducible	High metabolite detection
	Can be used for any sample	Can be combined with chromatography
General limitations	Moderate resolution due to signals overlap	Destructive method
	Low sensitivity (μg)	Moderate reproducibility with salty mixtures
	Low metabolites detection	Detection bias (positive or negative mode)
	Very expensive	

[a] See references [23,24,26–28,30] for more details.

strategy of choice for metabolomics [23,27]. MS can also be combined with chromatographic methods to increase metabolite separation [27–29]. Detailed information about the analytical aspects of metabolomics is available elsewhere [23,24,26–28,30].

Organ transplantation is considered as a complex pathophysiological condition that requires the monitoring of the graft-recipient's immunological interactions, graft function recovery, infection and immunosuppressant treatment. Metabolomics has the potential to identify biomarkers associated with the different operational stages and medical states of organ transplantation and was increasingly applied to study the dynamics of organ transplantation and immunosuppressants toxicity [3,31–33]. We will review the potential of metabolomics at the pre-transplantation stage in assessing general health/disease state of donors and recipients as well as organ quality monitoring [3,34]. The monitoring of warm and cold ischemia time and reperfusion injuries on the organs will be discussed as well [3,35], Finally, the application of metabolomics in the follow-up of graft function recovery, alloimmune rejection and immunosuppressant toxicity will be covered [3,36–39].

2. Metabolomics and organ transplantation

Organ transplantation is a complex procedure where early detection of organ injuries caused by rejection or infection processes is needed [3]. Currently, the diagnostic tools available to evaluate the nature and the severity of the pathologies associated with transplantation are either non-specific or invasive. Furthermore, considering the multifactorial aspect of graft dysfunction, metabolomics represents a promising strategy for graft follow-up. As shown in Table 2, many interesting studies were published in regard of various transplanted organs such as kidney, liver, heart, lung, corneal and intestines [2,3,32,33].

3. Metabolomics and kidney transplantation

3.1. Acute rejection of kidney allograft

The incidence of acute rejection (AR) has considerably decreased in the last decades, mainly by the use of more effective immunosuppressive agents [80]. Acute renal allograft rejection is a complex condition that can be triggered by infections, interstitial nephritis or acute tubular necrosis and can lead to graft loss or permanent impaired graft function [81]. Histologically, AR is characterized by tubulitis caused by the infiltration of lymphocytes in renal tubules and into the interstitium or by glomerulitis and peritubular capillaritis like evidenced by the presence of mononuclear cell into the capillary lumen or cellular infiltrates into the vessel walls [81]. Despite new acute kidney injury biomarkers, identification of specific circulating biomarkers is therefore highly required since the current monitoring is mainly achieved by serum creatinine measurement, which is non-specific, occurs late after the initiation of rejection process and must be monitored over long periods. Moreover, the diagnosis of rejection is only achieved by invasive kidney biopsy [81,82].

Exhaustive metabolomics studies aimed to characterize the metabolic changes in human serum during the AR process [46,47]. A large number of metabolites, including several amino acids, carbohydrates, lipids, gut microbiota-associated metabolites and catabolic compounds undergo significant concentration variation during AR [46,47]. Interestingly, one of these altered metabolites associated with AR is kynurenine [45,47]. Kynurenine is a metabolite of tryptophan degradation produced either by tryptophan 2,3 dioxygenase (TDO) or by indoleamine 2,3-dioxygenase (IDO) [83]. Interestingly, IDO was shown to potentially act as an immunomodulatory enzyme by inhibiting T cell proliferation and promoting immune tolerance [84–88].

Furthermore, several metabolomics studies were performed to characterize the metabolic patterns associated with AR in urine samples [44–47,89,90]. By applying a MS metabolomics approach, Wang et al. established a metabolic profile of 4 unidentified metabolites that predict AR with a 100% specificity when tested on 39 specimens [90]. Similarly, another group identified metabolites that are associated with T cell-mediated rejection (TCMR), in pediatric patients [45]. They identified 134 metabolites with altered levels from which the top 10 metabolites, that include kynurenine, two phosphatidylcholines and several amino acids and amino acids derivatives, were used to build a statistical model that predicts TCMR with 83% sensitivity and specificity [45]. One of the metabolites that displayed the most increased urinary levels was proline [45]. It was shown that proline is excreted by activated arginase-1 expressing macrophages, suggesting that altered metabolites levels in AR are either expressed by the alloimmune system or altered metabolism of injured tissue [45,91]. Additionally, they also