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Review

Recent insights on the significance of transcriptomic and metabolomic analysis of male factor infertility

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ABSTRACT

Infertility is a worldwide reproductive health problem which affects approximately 15% of couples, with male factor infertility dominating nearly 50% of the affected population. The nature of the phenomenon is underscored by a complex array of transcriptomic, proteomic and metabolic differences which interact in unknown ways. Many causes of male factor infertility are still defined as idiopathic, and most diagnosis tends to be more descriptive rather than specific. As such, the emergence of novel transcriptomic and metabolomic studies may hold the key to more accurately diagnose and treat male factor infertility. This paper provides the most recent evidence underlying the role of transcriptomic and metabolomic analysis in the management of male infertility. A summary of the current knowledge and new discovery of noninvasive, highly sensitive and specific biomarkers which allow the expansion of this area is outlined.

Contents

Introduction

Infertility, defined as the failure of a couple to conceive after 12 months of unprotected sexual intercourse, is affecting approximately 10–15% of all couples worldwide [1]. In particular, male factor infertility contributes nearly 30–55% of all cases, and is often the most difficult form of infertility [2]. The high rates of male infertility are governed by a variety of causative factors, including environmental disruptors, genetic defects, physiological and endocrine failure, and testis pathologies [3–6]. Specifically, despite the fact that decades of efforts to elucidate the etiological mechanism and molecular pathway of male infertility have been initiated, 60–75% of these cases are idiopathic [7]. The incidence is usually accompanied by qualitative (asthenospermia, teratozoospermia, and necrospermia) and quantitative (azoospermia, cryptozoospermia, and oligoasthenozoospermia) abnormalities [6,8].

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Current diagnostic tool for male infertility involves the evaluation of basic semen analysis [9]. The predictive value for the forecast of pregnancy is insufficient, due to the limitations of assessment before advancing to treatment with an assisted reproductive technology (ART) [10]. The analysis is conducted based on the ejaculate for abnormalities of sperm number, diminished sperm motility, and sperm morphological abnormality [11]. This sheds light on the necessity to discover and define a more precise and robust male infertility biomarkers for the prediction of pregnancy rate [12].

Today, transcriptomic and metabolomic studies have been conceived with an expectation to identify the useful biomarkers for a better diagnosis of male infertility. Accordingly, transcriptomics which joins the words "transcript" and "genome", is the integrity of ribonucleic acid (RNA) expressions' regulation and functional analysis of transcriptome [13]. Transcriptomics represents a promising approach for the discovery of new biomarkers able to improve the management of male factor infertility, and comparison of complete sperm expression profiles (SEP) statistically. In contrast, metabolomics is the small and low molecular weight cellular metabolic product study [14], which can be either intrinsic, resulting from normal cellular physiology, or extrinsic, resulting from the influences of exogenously administered environmental agents. In the identification and quantification of useful metabolite biomarkers for male factor infertility, a combination of analytical, biochemical, and spectral analyses has been established to differentiate between the signatures of the metabolites for healthy control populations and infertile men [15].

The exploration of new clinical biomarkers in spermatozoa, seminal plasma, testicular tissue, blood or urine has emerged to be a sensitive and specific diagnostic and prognostic tool to permit the early detection and treatment of patients with male factor infertility. Of major interest, transcriptomics and metabolomics have been proposed to be a highly promising method for the identification of new biomarkers for the early detection and therapy of male infertility. More importantly, transcriptomic and metabolomic studies could underline the specific biomarkers in categorizing, diagnosing and treating male factor infertility from the bench to the bedside. In this sense, this paper attempts to postulate an initial platform to raise the unique implications of transcriptomics and metabolomics in male infertility. The comprehensive and up-to-date literature of transcriptomics and metabolomics in the field of human male factor infertility for the last five years (2009–2013) has been summarized and outlined, with special highlight emphasis on the new biomarkers' discovery, characterization, and the possible future clinical applications.

Transcriptomic biomarkers in male factor infertility

Transcriptomic is a rapidly expanding area which aims to comprehensively profile all information in the RNA pool within a cell or tissue [12]. Spermatozoon contains many specific types of RNAs, particularly messenger RNAs (mRNAs), micro RNAs (miRNAs), and piwi-interacting RNAs (piRNAs). The physiological significance of these paternal haploid genome includes the stabilization of nuclear envelope during prostamine transition, DNA sequences marking, and early embryonic development [16,17]. Today, transcriptomic approach has been recognized to be a powerful tool to unveil the pathogenesis of the male factor infertility. Prospective epidemiological study has consistently shown strong associations between the impaired expressions of RNAs with male infertility [18,19]. This section describes the role of transcriptome profiling studies based on microarray data in male factor infertility. Specifically, the expressions of RNA as important biomarkers in male factor infertility were explored, and their involvement in the impaired spermatogenesis was identified. The recent advance of transcriptomic approaches to the management of male factor infertility is summarized in Table 1.

Transcriptomic profiling of human spermatozoa

Sperm analysis following World Health Organization (WHO) guidelines is unable to explain the molecular causes of male infertility when basic sperm parameters are within a normal range and women do not present gynecologic pathology. Consequently, for the first time, Garrido et al. [20] have discovered the potential role of transcriptomic profiling of spermatozoa biomarkers as a diagnostic tool to evaluate the genes, sequences, and biologic processes between the infertile and fertile men. Sperm samples were collected from strictly selected infertile men who attended at the infertility clinic. Basic sperm analysis, sperm mRNA extraction and microarray experiments were performed in duplicates, followed by the determination of biological functionality and mRNA levels for multiple genes. From the study, 50 spermatozoa transcriptomes were found to be differentially regulated between the fertile and infertile men, and trypsin X3 (TRY1), gamma-glutamyltransferase 1 (GGT1) transcript variant 3 and calcium-binding protein 39-like (CAB39L) genes in infertile men were over-expressed by ten times and more. Further bioinformatic analyses suggested that these over-expressed genes are likely to be involved in the biochemical and physiological pathways of male gamete formation or maturation. The study shed light on the possibility that male factor infertility may not be related to sperm production in terms of sperm count, but is significantly associated with the sperm function.

Meanwhile, Gatta et al. [21] have performed the testis transcriptome analysis on the pathogenesis of oligoazoospermia in cases with and without azoospermia factor c (AZFc) microdeletion. Thirteen patients carrying an AZFc microdeletion within the long arm of Y chromosome (Yq) or affected by idiopathic infertility have participated in this study, and the testicular phenotype was classified as either Sertoli cell only syndrome (SCOS), hypospermatogenesis (HS) or AZFc-b2/b4 deletion. Bilateral testicular biopsy was undertaken to examine the histopathology of the testis parenchyma. The total RNA was extracted, quantified, amplified fluorescently, labeled with Cy3–Cy5 cyanins, and hybridized on the high-density array. Hierarchical clustering was performed to classify genes based on their biological functions, and disclose the functional networks connecting these specific genes. The patients showed a down-regulation of several genes related to spermatogenesis, particularly those involved in testicular mRNA storage (AFF4, CCT6B, CRISP2, DDX25, FKBP6, KIF6, PRM2, SPAG6, TNP1, TSGA10, CLGN, DAZ1, TNP1, PCSK4, ARL4A and PTDSS2). Surprisingly, the idiopathic patients showed no testicular expression of DAZ, an important gene which is involved in the translational regulation of gene expression, despite the absence of AZFc deletion in the peripheral blood. The results supported a possible intriguing hypothesis that DAZ gene dysfunction can translate to a pathogenic defective mRNA storage in the testis with great pathogenic implications, and it can account for a large number of previously thought "idiopathic" infertility. Substantial to this finding, investigation on DAZ gene dysfunction is essential to reveal the molecular determinants of infertility that are undetected when only testing Yq deletions in the peripheral blood.

MicroRNAs (miRNAs) are short (20–24 nucleotides), single stranded noncoding RNA molecules, which functions in transcriptional and post-transcriptional regulation of gene expression [22,23]. Alterations of miRNA expression and mutation have been related to spermatogenic failure [24–26]. A descriptive study was conducted in Colorado to describe for the first time that aberration of blastocyst miRNA expression was associated with human infertility, including the male factor infertility [27]. The miRNAs from each blastocysts were isolated, reverse transcribed and quantified for a set of three miRNA predicted targets, namely ARIH2 (predicted target gene for miRNA hsa-miR-19a), KHSRP and NFAT5 (predicted target genes for miRNA hsa-miR-24). The relationship between miRNA expressions with the male factor infertility was established. The expressions of two miRNAs, Hsa-let-7a and has-miR-24, were found to be significantly decreased among patients with male factor infertility. Annotation of the gene targets for hsa-let-7a and hsa-miR-24 revealed mutual gene ontology (GO) biological