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Circulating microRNA-320a and microRNA-486 predict thiazolidinedione response: Moving towards precision health for diabetes prevention

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ABSTRACT

Introduction. The aims of this study were to compare microRNA (miR) expression between individuals with and without insulin resistance and to determine whether miRs predict response to thiazolidinedione treatment.

Materials and methods. In a sample of 93 healthy adults, insulin resistance was defined as steady state plasma glucose (SSPG) ≥ 180 mg/dL and insulin sensitive as <120 mg/dL. Response to thiazolidinedione therapy was defined as ≥10% decrease in SSPG. We selected a panel of microRNAs based on prior evidence for a role in insulin or glucose metabolism. Fold change and Wilcoxon rank sum tests were calculated for the 25 miRs measured.

Results. At baseline, 81% (n = 75) of participants were insulin resistant. Five miRs were differentially expressed between the insulin resistant and sensitive groups: miR-193b (1.45 fold change (FC)), miR-22-3p (1.15 FC), miR-320a (1.36 FC), miR-375 (0.59 FC), and miR-486 (1.21 FC) (all $p < 0.05$). In the subset who were insulin resistant at baseline and received thiazolidinediones (n = 47), 77% (n = 36) showed improved insulin sensitivity. Six miRs were differentially expressed between responders compared to non-responders: miR-20b-5p (1.20 FC), miR-21-5p, (0.92 FC), miR-214-3p (1.13 FC), miR-22-3p (1.14 FC), miR-320a (0.98 FC), and miR-486-5p (1.25 FC) (all $p < 0.05$).

Discussion. This study is the first to report miRs associated with response to a pharmacologic intervention for insulin resistance. MiR-320a and miR-486-5p identified responders to thiazolidinedione therapy among the insulin resistant group.

1. Introduction

Nearly 40% of insulin resistant individuals will develop type 2 diabetes [1] the majority of individuals with type 2 diabetes are insulin resistant [2]. Insulin resistant individuals are also at increased risk for a number of other serious diseases, including cardiovascular disease, certain types of cancer, polycystic ovarian syndrome, and non-alcoholic fatty liver

Abbreviations: miR, microRNA; TZD, thiazolidinedione; HDL-c, high density lipoprotein cholesterol; RT-PCR, reverse transcription polymerase chain reaction.

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[3–5]. In the absence of a simple way to identify individuals who are insulin resistant [6], clinical benefit may be realized if circulating microRNAs (miRs) can identify insulin resistant persons before the development of frank disease.

Recent studies showed differential expression of circulating miRs in individuals with type 2 diabetes compared to healthy controls [7–10]. MiR-126 is associated with 10-year risk for developing type 2 diabetes [10]. Levels of miR-126 were also observed to decrease in a graded fashion from normal glucose tolerance to impaired fasting glucose to type 2 diabetes [7]. Furthermore, circulating miRs are able to differentiate between individuals with type 2 diabetes who are normal versus overweight [8]. These findings support the possibility that circulating miRs may reflect underlying heterogeneity in the etiology of type 2 diabetes and function as predictors of incident type 2 diabetes.

A second potential clinical application of miRs is the identification of individuals who will respond to insulin sensitizing interventions. Thiazolidinediones (TZDs) are unique pharmacologic agents that improve insulin sensitivity in insulin resistant individuals without type 2 diabetes [11–15]. With optimal treatment, 1.0–1.5% improvements in glycosylated hemoglobin and concurrent improvements in serum lipids and lipoproteins are achieved [15]. Although TZDs are effective, there are potential adverse consequences and inter-individual differences in response to TZDs [16]. As a result, this class of pharmacologic agent has extremely limited use in clinical practice. Identification of individuals who are (1) responders likely to see improvement in insulin sensitivity, and (2) high risk for adverse events could provide sufficient information to allow reintroduction of these effective drugs into clinical practice.

In response to these issues, this study was designed to address two aims. The first was to compare miR expression between non-diabetic individuals with and without insulin resistance determined by a direct measure of insulin-mediated glucose disposal by the insulin suppression test. The second was to determine whether miRs were associated with improved insulin sensitivity following TZD treatment. The findings of this study provide new information about the identification of circulating miRs as potential biomarkers for insulin resistance and identify miRs that predict which individuals will exhibit improvements in insulin sensitivity following TZD therapy. This study serves as an exemplar for the development of individualized treatment approaches to optimally decrease risk for type 2 diabetes.

2. Materials and Methods

2.1. Study Design, Setting, and Sample

Participant samples and data for the current study were collected during prior studies evaluating the effect of TZDs in generally healthy individuals without type 2 diabetes. The study designs were reported in detail previously [13,14,17]. Participants were a multi-ethnic group of male and female adults recruited by convenience from the community around the Stanford University Medical Center. All three studies had a single intervention arm design to test either the effects of rosiglitazone on insulin secretion and clearance [14] and vascular and inflammatory markers [13] or pioglitazone on cardiovascular risk factors [17]. Participants in all studies received either 4 mg rosiglitazone for 4 weeks followed by 8 mg rosiglitazone for 8 weeks (n = 55) [13,14] or 15 mg pioglitazone for two weeks then 30 mg pioglitazone for 2 weeks followed by 45 mg for 8 weeks (n = 38) [17]. From these studies, we included only individuals who were in the lowest or highest tertile of insulin resistance at baseline (n = 93). The subset who were insulin resistant at baseline and received TZDs (n = 47) were evaluated for TZD response. All studies were approved by the Stanford University Institutional Review Board.

2.2. Clinical Data Collection

Measurement of demographic and clinical variables, insulin sensitivity, and collection of blood plasma was done during the baseline visit by trained personnel in the Stanford University Medical Center Clinical Research Center. Total cholesterol, high-density lipoprotein cholesterol (HDL-c), and triglycerides were measured by the vertical auto profile II method [18]. Insulin-mediated glucose disposal was quantified by a modification [19] of the insulin suppression test [20]. After an overnight fast, subjects were infused for 180 minutes with octreotide (0.27 $\mu g/m^2$/min), insulin (32 mU/m^2/min), and glucose (267 mg/m^2/min). Plasma glucose and insulin were measured every 10 minutes during the 150- to 180-minute period, and then averaged to determine steady state plasma glucose and steady-state insulin concentrations. Insulin resistance was defined as the lowest tertile of insulin sensitivity (steady state plasma glucose (SSPG) $\geq$ 180 mg/dL) and insulin sensitivity as the highest tertile of insulin sensitivity (SSPG < 120 mg/dL).

2.3. Molecular Data Collection at Baseline

Blood used for banking of plasma was collected at baseline through an intravenous catheter during the insulin suppression test protocol. Blood was collected into vacutainers containing the preservative EDTA, centrifuged at 4 °C to separate plasma from cellular blood components, and stored at −80 °C. Samples were collected between January, 2001 and August, 2008. RNA was isolated from plasma using Trizol and eluted in nuclease free water. We selected a panel of 25 miRs to measure based on previous data from human studies of blood-based expression of miRs in type 2 diabetes and related conditions [10,21–26]. Twenty-one miRs (i.e., hsa-miR-122-5p, hsa-miR-126b-3p, hsa-miR-133a-3p, hsa-miR-144-3p, hsa-miR-146a-5p, hsa-miR-146b-5p, hsa-miR-150-5p, hsa-miR-182-5p, hsa-miR-191-5p, hsa-miR-193b-3p, hsa-miR-197-3p, hsa-miR-20b-5p, hsa-miR-21-5p, hsa-miR-223-5p, hsa-miR-27a-3p, hsa-miR-29b-3p, hsa-miR-320a, hsa-miR-34a-5p, hsa-miR-370-3p, hsa-miR-375, hsa-miR-486-5p) were selected based on biological plausibility. The remaining four (i.e., hsa-miR-133b, hsa-miR-214-3p, hsa-miR-22-3p, hsa-miR-296-5p) were selected as potential normalizers based on studies showing low variability of these species in studies of human plasma [10] and whole blood [27].

Initial measurements were done using the Firefly Circulating miRNA Assay (Firefly BioWorks, Cambridge, MA) [28]. MiRs were hybridized to complementary oligonucleotides covalently attached to encoded hydrogel microparticles. The