A panel of blood-based circulatory miRNAs with diagnostic potential in Psoriasis patients

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Abstract

Objective To explore a set of inflammation associated circulatory miRNAs with potential role in etiopathology of psoriasis as differential disease biomarker/s.

Subjects and Methodology Expression profiling of 15 immunologically relevant miRNA candidates was done on plasma samples from psoriasis vulgaris patients compared to healthy subjects by qRT-PCR. Receiver operating characteristic (ROC) curve was analyzed for significantly altered miRNAs to evaluate their disease diagnostic potential. Correlation analysis among significantly altered miRNAs and their association with disease severity (PASI) was done to validate the candidates as robust disease biomarker/s. Downstream targets and the pathways for each of the significant miRNAs were deciphered using miR target prediction tools and pathway enrichment to elucidate their functional relevance in disease pathogenesis.

Results: 5 miRNAs exhibited a significant change in their expression level among the 15 candidates tested viz miR-215, miR-148a, miR-125b, miR-223 and miR-142-3p. ROCs for all the 5 miRNAs individually as well as in combination exhibited a significant disease discriminatory area under the curve, AUC = 0.762 and p< 0.0001 for all the miRNAs taken together. Statistically, all 5 miRNAs in combination depicted a robust model as disease severity correlate compared to individual miRNAs with highest R² value = 0.94 and least AIC score =131.8. Each of the miRNA also exhibited a significant association with at least one of the other miRNAs in the panel. All the 5 miRNAs exhibited functional role in psoriasis immune-pathogenesis in terms of their downstream targets and signaling pathways.

Conclusion: In summary our findings identify a panel of blood-based circulatory miRNAs comprising miR-215, miR-148a, miR-125b-5p, miR-223 and miR-142-3p that can differentiate psoriasis vulgaris patients from healthy individuals. In light of the role of each of these miRNAs in modulating immune-inflammatory pathways, the panel provides a rationalized combination of biomarkers that can be tested further on an expanded cohort of patients for its diagnostic value.

Introduction

Psoriasis is a chronic inflammatory skin disease with itchy and painful cutaneous manifestations, affecting nearly 2% of the world's population [1, 2]. Psoriatic lesions are characterized by abnormal differentiation and hyperproliferation of keratinocytes with immune cell infiltration. Disease etiology involves a complex interplay between genetic and environmental factors such that the homeostatic cross talk between the skin keratinocytes and different immune cells normally seen in healthy skin is disturbed. Despite substantial studies on understanding the initiation and progression of disease, exact molecular mechanisms that dysregulate the complex interactions among the lesional cells to create a chronic inflammatory environment are not fully known. Also, significance of systemic immune changes associated with the disease is not much understood [1]. Immunologically, new insights in the pathogenesis of psoriasis imply a major role of T cells in the initiation and maintenance of the inflammatory state which can potentially lead to keratinocyte specific changes seen in the disease lesions [3, 4]. Generally, the disease is diagnosed by clinical evaluation of skin lesions by expert dermatologists, with occasional histopathological examination [5]. With no reliable molecular biomarkers as criteria for clinical diagnostics till date, there is an urgent requirement of differential biomarker/s with diagnostic potential and predictive value.

Psoriasis is an outcome of several genetic-epigenetic, environmental and immunological factors wherein recently miRNAs have emerged as critical regulators of disease pathogenesis [6-11]. miRNAs are small ~ 20-25 bp non-coding RNAs that control gene expression in a cell specific manner with wide functional implications in processes like development, growth, apoptosis, plasticity, activation, survival, proliferation and differentiation. In this context miRNAs that regulate keratinocyte and heterogenous T cell population become significant in pathophysiology of psoriasis [12-16]. Although produced within cells, miRNAs with altered cellular expression have been shown to be mirrored as deregulated stable cell free molecules in peripheral circulation under disease condition [17, 18]. Thus, disease specific circulatory miRNA pool makes it amenable to develop minimal invasive blood based diagnostic biomarkers in relation to disease prediction and severity. Several studies on miRNAs expression analysis have been carried out across various sample types viz blood, PBMCs, hair and lesional skin for potential diagnostic in psoriasis [19-22]. Explorations on differentially expressed systemic miRNAs in sera/plasma samples with select miRNA candidates have been done with few studies based on human miRNA array and small RNA sequencing analysis [19, 20, 22,27]. Heterogenous findings on miRNA candidates with respect to disease specific differential expression, low abundance, non-significant correlation with disease severity, lack of knowledge on role of altered miRNAs in disease progression, small sample size used, variation in blood component used as samples in these studies are limiting towards transition to their clinical use.

In the present study, a set of immunologically relevant, T cell associated miRNA candidates have been selected to access their differential expression in plasma of psoriatic patients [28-35]. With potential to drive inflammatory disease processes, we have deciphered a combination of 5 significant miRNAs that can provide a robust diagnostic panel in light of their functional implication in disease etiology and progression.

Materials And Methods

Study subjects and sample collection

A total of 40 psoriasis vulgaris patients clinically confirmed by dermatologists along with sex-matched 40 healthy subjects were enrolled in the study. The patients with psoriasis were recruited from the outpatient dermatology clinic at the Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab, India and All India Institute of Medical Sciences, Bathinda, Punjab, India. Patients 18 years of age or above, with a clinical diagnosis of mild to severe psoriasis vulgaris (PV), who have not received topical therapy for at least 2 weeks, systemic immunosuppressive treatment or Phototherapy, for at least 1 month were included while psoriasis patients with other infections or co-morbidity were not enrolled in the study. Disease severity was graded by the psoriasis area and severity index (PASI) score and body surface area (BSA) into mild (PASI score ≤ 5/BSA ≤ 3%), moderate (PASI score 5-10/BSA 3-10%) and severe (PASI score >10/BSA > 10%) [36]. 5 mm punch biopsies were taken from lesional skin of 16 patients. Non-lesional biopsies from the same 16 patients from the uninvolved skin away from the lesion was also collected for comparative analysis. Healthy age-sex matched controls were recruited from the staff and graduate students...
at the Central University of Punjab, Bathinda, Punjab, India. Written informed consent along with subject information form was obtained from all the participants. The present study was conducted in accordance with the declaration of Helsinki and approved by the Ethics Committee of Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab, India (GGS/IEC/19/57), AIIMS, Bathinda, India (IEC/AIIMS/BTI/150) and Central University of Punjab, Bathinda, Punjab, India CUPB/IEC/2016/045).

Sample Processing

Lesional and non-lesional tissue from 16 psoriasis patients were processed for disease specific histopathological examination out of total 40 patients enrolled in the study. 5mm lesional and non-lesional punch biopsies were collected and fixed in 10% neutral buffered formaldehyde (NBF). Paraffin embedded tissue was processed into 5 µm sections, stained with Hematoxylin and Eosin (H&E) for histopathological analysis. Whole blood samples from 40 psoriasis patients and 40 healthy controls were collected in EDTA-coated vials. The blood samples were centrifuged at 1800 rpm for 10 min at RT to collect the straw-colored platelet rich plasma. The plasma was centrifuged again at 2000 rpm for 10 min at 4°C to remove the remaining cellular components. The purified plasma samples were stored in aliquots at -80°C until further assays. 200 µl of each plasma sample was used for miRNA extraction.

Circulating miRNA isolation, cDNA synthesis and qRT-PCR assay

miRNA extraction from plasma samples was done using miRNeasy serum/plasma kit (Catalogue no. 217184: Qiagen Inc USA,) according to the manufacturer's instructions. The concentration and purity of miRNA was determined with Nanodrop UV-Vis Spectrophotometer 2000cc (Thermo Fisher Scientific, CA, USA). 100ng of total isolated miRNA was used for cDNAs synthesis, using miScript II RT kit following manufacturer's protocol (Catalogue no. 218161: Qiagen Inc. USA). A set of 15 immunologically relevant miRNAs with respective sequences enlisted in Table S1 were quantified in plasma samples of psoriasis patients compared to health cohort by real time PCR using Qiagen miScript SYBR Green PCR kit and miR specific primers (Catalogue no. 218073: Qiagen Inc. USA, Cat. no. MS00004900; Qiagen Inc, Quant studio 3 Applied biosystems real-time PCR). Cel-miR-39 was used as an internal control. qRT PCR reaction comprised of 6.25µl SYBR green master mix, 1.25µl each of miR specific primer and universal primer and 3.5µl of nuclease free water with required amount of cDNA. Reaction conditions were as follows. Initial activation done at 95°C for 15 min, denaturation at 94°C for 15 sec, annealing at 55 °C for 30 sec, extension at 70°C for 30 sec for 40 cycles with melting curve analysis for quality check. A non-template control reaction for each primer was also carried out to check for contamination by including all components except for the cDNA template. Threshold cycle (Ct) value for each sample with primers was recorded. The relative expression between psoriasis patients and healthy controls was estimated by the 2−ΔΔCt method [37] where ΔΔCt = sample's average Ct value for specific miR – sample's average Ct value for Cel-miR-39 [38] . All reactions were repeated at least three times.

Network Analysis of miRNA Target Pathways

Target genes for five miRNAs of interest viz miR-215, miR-142-3p, miR-223, miR-148a and miR-125b-5p were predicted using four different miRNA target prediction tools: miRDB (http://www.mirdb.org/), TargetScan (https://www.targetscan.org/vert_80/), DianamicroT(https://diana.imis.athenainnovation.gr/DianaTools/index.php?r=MicroTCDS/index), Mirabel (http://bioinfo.univ-rouen.fr/mirabel/). For each miRNA, common targets based on the four databases were deciphered. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were enriched for each of the miRNAs using Starbase (https://starbase.sysu.edu.cn/). The enriched pathways were analysed for the signaling components overlapping with the common set of targets for each miRNA retrieved from the four databases. The significant pathways involving miRNA specific targets were reviewed for the potential role in T cells and/or keratinocyte associated biological functions and were mapped for interaction network using Cytoscape.

Statistical Analysis

Non-Parametric Mann Whitney-U test were performed to compare the qRT PCR based miRNA expression differences in psoriasis patient group and age, sex matched healthy control group. Receiver operating characteristic (ROC) curves were generated with calculation of area under the curve (AUC) as an index for evaluating the diagnostic potential of miRNAs of interest to differentiate psoriasis patients from healthy controls [39]. A p-value ≤ 0.05 was considered statistically significant for qRT-PCR data and ROC analysis. All statistical calculations were performed using GraphPad prism 8.0.2 and R software 4.2.2. Pearson correlation coefficients were derived to study the pairwise relationship between expression levels of each of the miRNAs among study subjects (psoriasis patients and control subjects). A p-value ≤ 0.05 was considered statistically significant. Non-linear model b-spline was used to study the individual as well as joint impact of miRNA levels in relation to the PASI score.

Results

Study Cohort

A total of 40 patients clinically confirmed for psoriasis vulgaris and 40 healthy subjects enrolled in the study were grouped into age and sex matched study cohort. Patients group including males as well as females comprised of clinically identifiable individuals with typical dry, raised, erythematous psoriatic plaques exhibiting silvery scales and were graded as mild, moderate and severe based on disease severity with PASI score ranging from 3.5-15.6 and BSA from 2%-58% (Fig 1 Panel A, Table 1). 5 mild, 18 moderate and 17 severe psoriasis patients with no ongoing treatment at the time of diagnosis and sample collection were enrolled for experimental studies. Histopathological analysis of lesional versus non-lesional tissue was done for biopsies from16 patients. Hematoxylin & Eosin-stained skin lesional sections exhibited psoriasis specific dermal and epidermal changes to variable extents (Fig 1 Panel B, Table 1). All the 16 patients exhibited thickening of the epidermis designated as acanthosis with severe manifestation leading to elongation of rete ridges in 12 patients out of 16. Hyperkeratosis with thickened stratum corneum, parakeratosis with abnormal retention of nuclei in the stratum corneum layer and dermal leukocyte infiltrates were apparent in 13 specimens with hypgranulosis in 12 patients. An exploration of the systemic expression profile of miRNAs of interest was further done on the study cohorts.
Expression pattern of select plasma miRNAs

A set of 15 immunologically relevant miRNA candidates were explored for qRT based differential expression in plasma samples of 12 psoriasis patients and age/sex matched 12 healthy controls (S Table 1). 5 miRNAs viz miR-215, miR-148a, miR-125b, miR-223 and miR-142-3p exhibited an expression pattern with consistent and significant changes. miR-215 was significantly decreased (Fold change = 2.24, \( p\text{-value} = 0.043 \)) while four miRNAs miR-148a, miR-125b, miR-223 and miR-142-3p were significantly upregulated (Fold change = 1.82, 1.84, 2.42 and 2.56 and \( p\text{-value} = 0.032, 0.034, 0.028, 0.045 \) respectively) in psoriasis patients compared to healthy controls. miR-146a and miR-21 showed a trend towards upregulation with no significant change while no change was seen in miR-155 expression levels. The remaining miRNA candidates miR-590-5p, miR-15b, miR-568, miR-150, miR-23b, miR-27b, miR-184 were associated with high sample to sample variation in terms of threshold detection or low abundance. The five miRNAs with significant dysregulation in expression levels were further validated in an extended cohort of patients and healthy study subjects. In line with initial results, cumulative study cohorts with 38 patients versus 38 healthy subjects exhibited significant downregulation in miR-215 expression (Fold change = 3.34, \( p\text{-value} = 0.001 \)) and significant upregulation in miR-148a (Fold change = 2.43, \( p\text{-value} < 0.05 \)) and miR-125b (Fold change = 1.75, \( p\text{-value} < 0.05 \)) and miR-223 (Fold change = 2.79, \( p\text{-value} < 0.001 \)) and miR-142-3p (Fold change = 2.27, \( p\text{-value} < 0.01 \)) as shown in Fig 2, Panel A and Table 2. The five miRNAs of significance were tested for their diagnostic potential to differentiate psoriasis vulgaris patients from healthy individuals.

miR-215, miR-148a, miR-125b, miR-223 and miR-142-3p constitute a potential diagnostic panel for Psoriasis vulgaris

Receiver operating characteristic (ROC) curves for each of the significantly altered miRNAs were generated and area under the receiver operating characteristic curve (AUC) was employed as an accuracy index for evaluating the diagnostic performance of the 5 miRNAs with significant expression changes. ROC analysis of miR-215, miR-148a, miR-125b, miR-223 and miR-142-3p corresponded to AUCs of 0.873, 0.768, 0.709, 0.790 and 0.787 respectively implying a robust discriminatory potential of each of the miRNAs (Fig 2 Panel B, Table 2). The combination of all the five miRNAs together also exhibited a significant disease discrimination readout with AUC = 0.762, \( p\text{-value} < 0.0001 \) (Fig 2, Panel C).

Correlation analysis between each of the five miRNAs for the control and psoriasis subjects was done to explore association between their expression patterns (Fig 3a, Table 3). Based on Pearson correlation coefficient as a readout, each of the five miRNAs exhibited significant correlation with atleast one of the miRNAs in the panel. miR-215 showed negative correlation with all other 4 miRNAs and significant association only with miR-223 (\( p\text{-value} < 0.05 \)). miR-148a, miR-142-3p, miR-223 and miR-125b showed a trend towards a positive correlation between each other with significant positive correlations between miR-148a and miR-142-3p (\( p\text{-value}<0.05 \)), miR-142-3p and miR-223 (\( p\text{-value} < 0.05 \)), miR-223 and miR-125b (\( p\text{-value} < 0.05 \)). AUC values along with correlation matrix imply the miRNA panel with the combination of a downregulated miR-215 and upregulated miRNAs miR-148a, miR-125b, miR-223 and miR-142-3p as a promising set of diagnostic miRNA biomarkers for psoriasis vulgaris patients.

Correlation of miRNA expression with clinical severity

All the five miRNAs were further analysed for their correlation with disease severity in terms of patient PASI score, alone or in combination. With a non-linear association pattern derived between miRNA expression levels and PASI score, b-spline model was used to decipher their relationship. Based on best fit b-spline model, all the five miRNAs together exhibited the best fit model with \( R^2 \) value = 0.94 and lowest AIC score of 131.8 (Fig 3b). None of the individual miRNAs showed a good fit with PASI score with lower \( R^2 \) values and higher AIC readouts. Individually, miR-215 alone showed the highest correlation (\( R^2 \) value = 0.56 and AIC = 162.3) compared to each of the other miRNAs. The data imply that all the five miRNAs in combination as a panel showed a robust correlation with the disease severity unlike individual miRNAs.

Understanding Candidate miRNA Target Pathways with functional implication in Psoriasis

Enrichment of significant pathways involving direct targets of deregulated panel of 5 miRNAs comprising miR-215, miR-142-3p, miR-125b, miR-148a and miR-223 was performed using a combination of the miRNA specific targets and KEGG pathways as detailed in material and methods section. A set of signaling pathways downstream of one or more than one miRNA were delineated in light of literature as shown in Table 4. All the relevant targets involved in the enriched pathways were mapped as a miRNA-mRNA regulatory network. The panel of 5 deregulated miRNAs exhibited a network of interconnected targets as part of one or more enriched pathways of interest, color coded in Fig 4 with supporting information in Table 4. All the relevant targets involved in the disease severity were upregulated and AIC = 162.3) compared to each of the other miRNAs. The data imply that all the five miRNAs in combination as a panel showed a robust correlation with the disease severity unlike individual miRNAs.

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Multiple chemokines produced by keratinocytes and immunocytes potentially contribute to the inflammatory psoriatic lesions and systemic changes involving direct targets of miR-125b and miR-148 [62]. Stat3 as a direct target of miR125b and downstream of multiple chemokine/cytokine inflammatory pathways has been shown to dysregulate keratinocyte proliferation and apoptosis [63]. T cell receptor signaling constitutes the primary event for a homeostatic effector T cell function which is modulated in autoimmune condition of psoriasis with a pathogenic T cell response [64, 65]. The perpetual T cell activation drive the keratinocyte inflammatory loop with manifestation of psoriatic hyperplasia [66]. miR-125b-5p and miR-148 regulate multiple targets directly involved in T cell receptor signaling [43, 45]. IL-21, a T cell derived cytokine leads to epithelial hyperplasia in psoriasis by inducing keratinocyte proliferation [67]. IL-21 signaling regulate T cell activation, proliferation, and differentiation [68]. Among our miRNAs of interest miR-215 targets IL-21 transcript [69]. IL-17 signaling play a major role in setting the inflammatory pathophysiological changes associated with psoriasis with enhanced IL-17 producing T cells and inflamed hyper-proliferative keratinocytes in response to amplified IL-17 expression in psoriatic lesions and systemic circulation [70, 71]. IL-17 signaling components and effector molecules viz IL-17RA, IL-17RE, RUNX1 show up as direct targets of miR-215 [72, 73]. Altered cell cycle progression and apoptosis in an inflammatory skin micro-environment is a hallmark of psoriatic lesions with epidermal hyperplasia [44, 45, 51, 74, 75]. Specific molecules involved in these cellular processes comprise direct targets of miRNAs of interest. The miR-215-XIAP axis alters cellular apoptosis while miR-142-3p targets E2F7 and E2F8, the transcription factors that regulate T cell cycling [76-78]. All the five miRNAs with significant expression changes in peripheral circulation showed downstream target pathways with functional role in keratinocyte and/or T cells implying their potential role in etiology of Psoriasis.

Discussion

Psoriasis is a cutaneous manifestation with T cells as crucial mediators of lesional and systemic inflammatory changes. Disease diagnosis is majorly dependent on clinical identification of typical psoriatic lesions in conjunction with histopathological findings. Till date, there is unavailability of reliable molecularly defined diagnostic markers for the disease in spite of substantial knowledge on key cellular and molecular processes that define psoriasis. In this context, miRNAs representing specific and stable small non-coding RNAs involved in pathophysiology of psoriasis comprise a promising set of molecules with diagnostic potential. A large number of such studies on differential expression of miRNAs in varied sample types from psoriasis patients have been performed to elucidate their diagnostic and prognostic value. Limitations with respect to significant differential expression, heterogenous abundance and correlation with disease severity, lack of knowledge on role of altered miRNAs in disease progression, small sample test size, variable sample types used are in the way of translating these findings to clinical use.

In the present study 15 immune-miRNAs were tested with the rationale of their functional involvement in T cell mediated immune-inflammation associated with lesional and systemic psoriatic changes. Out of all the miRNAs, five candidates showed significant differential expression with downregulation of miR-215 and upregulation of miR-148a, miR-223, miR-142-3p, miR-125b-5p. All the 5 miRNAs exhibited potential for differential diagnostic of psoriasis patients as per significant and acceptable AUC values for each of the 5 miRNAs individually as well as a combined panel [39].

The expression pattern data of the five significant miRNAs and their correlation trend among each other and with PASI score as a panel signify a promising combination of miRNAs with diagnostic value. Further, the nature of significant miRNAs deciphered in the present study with 2 newly profiled miRNAs (miR-215 and miR-148a) and 3 previously studied miRNAs (miR-223, miR-142-3p, miR-125b-5p) such that miR-215 is downregulated and the other four miRNAs are upregulated presents a robust miRNA combination that should be tested for differential diagnosis in a larger cohort of psoriasis vulgaris patients in a clinical setting. Across the different exploratory searches for circulatory diagnostic miRNAs that include global miRNA expression profiling and/or miRNA candidate specific expression studies, this is the first report on a panel-based set of miRNAs that can be taken up further as biomarkers for diagnosis of psoriasis vulgaris [19, 20, 22-27].

Interestingly, all the five miRNAs exhibited a validated and/or predicted role in dysregulating T cell and/or keratinocyte function, thus implying their involvement in inflammatory psoriatic manifestations. Altered expression of the 5 miRNAs in peripheral circulation found in our study may reflect their dysregulation in keratinocytes and/or T cells that constitute the key cell types involved in pathophysiology of the disease as depicted in Fig 5. Much of the literature on these miRNAs demonstrated specific targets and associated pathways involved in dysregulation of psoriatic keratinocytes albeit with limited knowledge on their T cell centric role in disease development.

miR-215 is associated with multiple cellular processes viz proliferation, apoptosis, migration, invasion, and epithelial-mesenchymal transition in various cancers [79, 80]. In Psoriasis miR-215 is specifically downregulated in the skin lesion tissues in humans as well as mice model with the functional role validated in keratinocyte specific study wherein miR-215 is shown to target Dyrk1A and dysregulate keratinocyte proliferation via EGFR signaling [6, 58, 81]. In context of T cells, miR-215 has been reported to be differentially expressed in Th2 subset in healthy human subjects with no T cell associated report in psoriasis [30, 82]. IL-17 receptors, RUNX1, IL-21 with miR-215 binding UTRs constitute Th17 subset specific signature molecules implying potential role of miR-215 in psoriasis associated Th17 dysregulation [69, 72, 73]. Also, not a single report on expression pattern in peripheral circulation in psoriasis patients is available on miR-215, possibly due to its low abundance along with its down regulated expression detected in our study. miR-148a has been demonstrated to contribute towards maintenance of a chronic inflammatory immune-environment by regulating persistence of activated Th1 cells in murine helper T cell model [83]. With upregulation in its expression in PBMCs from psoriasis patients, miRNA-148a has been shown to facilitate differentiation of inflammatory monocyte derived dendritic cells via PU.1-miR-148a-MAFB axis albeit with no report in context of psoriatic lesions, T cells and peripheral circulation [84]. miR-223 is another miRNA associated with pathogenic T cells in autoimmune diseases like Rheumatoid Arthritis and Multiple Sclerosis [85-87]. In context of psoriasis, miR-223 is reported as one of the important miRNAs altered in skin lesions with changes in epidermal, dermal infiltrate along with peripheral Th17 subset involved in inflammatory disease outcome [7, 81, 88]. Mechanistically, miR-223 has been shown to mediate keratinocyte hyperproliferation and apoptosis via PTEN/Akt pathway in a HaCaT cell line model [53]. Additionally, miR-223 is shown to be differentially upregulated in PBMCs with variable reports on extracellular systemic circulation in psoriasis patients. Lovendorf et al and Pivarcsi et al reported no change in psoriatic samples in two independent reports while Garcia et al demonstrated an increase in peripheral circulation similar to our finding in psoriatic plasma samples [20, 26, 89]. miR-142-3p is known to be a prominent hematopoietic miRNA with role in T cell cycling along with involvement in controlling T cell subset cAMP levels with functional implication in Treg
The miRNA is shown to be differentially detected in psoriatic skin miRNAome with differential expression in psoriatic epidermis, dermal infiltrate and peripheral T cells in the same patient cohort [7, 81, 88]. Mechanistically, miR-142-3p has been shown to dysregulate keratinocyte proliferation and apoptosis via targeting Sema3A based on HaCAT cell line studies [59]. Blood based studies on miR-142 exhibit variable findings with no change to a downregulated expression pattern unlike the significant increase found in plasma samples in our study [25, 26]. miR-125b constitute a signature miRNA responsible for maintenance of T cell naivety and thus regulate effector T cell function based on its high expression in naïve T cells in healthy individuals as well as in naïve T cells from psoriatic patients [30, 88]. The lesional miRNA profiling is also reported with miR125b as one of the most downregulated miRNAs in epidermal layer as well as in dermal infiltrates [6, 88]. The major cell type in psoriatic lesions with decreased expression is reported to be keratinocyte that exhibit miR-125b mediated hyperproliferation and abnormal differentiation via multiple signaling pathways validated in in vitro studies [47, 60, 63, 91]. Studies on serum expression demonstrate different findings with downregulation by Koga et al and Pan M et al with no change reported by Hernandez et al unlike significant upregulation detected in our study [19, 25, 60].

All the five miRNAs with their predicted or validated targets constituted regulators of one or more immune-inflammation associated pathways such that changes in their levels in keratinocytes and/or T cells can potentially lead to development of autoimmune-psoriatic manifestations. The miR-mRNA network analysis with predicted/validated targets highlights dysregulation of multiple auto-immune disease related pathways that may contribute to keratinocyte hyperproliferation and abnormal differentiation along with altered T cell activation, differentiation and effector function. All the pathways viz Wnt, MAPK, TGF-β, PI3K, mTOR, Notch, IL-21, IL-17, chemokine signaling, TCR signaling are reported to directly impinge on activation and proliferative capabilities of keratinocytes and T cells with elaboration of inflammatory milieu as the end result in disease development [41, 46, 49, 52, 56, 61, 62, 64, 68, 70].

Importantly, the five miRNAs together exhibited a significant correlation with the PASI score over individual candidates implying the combination of five miRNAs as a promising disease diagnostic panel. The high value of correlation of determination between PASI and all the five miRNAs taken together corroborates the involvement of multiple miR-target pathways that may drive the disease severity. In context of specific limitations in our study, each of the five miRNAs exhibited significant correlation with only one or two of the other miRNAs in the panel. This could be because of the heterogeneous expression of different miRNAs in the healthy as well as psoriasis patient subjects. An extended cohort of the control and diseased subjects with inclusion of more of severe psoriasis patients may give better relationship among the five miRNAs. Importantly, experimental validation of the downstream miRNA targets can further provide proof of concept of our insilico derived miRNA-mRNA regulatory network. Nonetheless, in light of existing literature, the plasma miRNAs viz miR-215, miR-142-3p, miR-223, miR-125b-5p and miR-148a constitute a promising panel of miRNA-based biomarkers potentially involved in a pathogenic inflammatory response as an outcome of a perturbed keratinocyte-T cell crosstalk.

Declarations

Compliance with ethical standards

Conflict of Interest

The authors declare that they have no competing interests.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional ethical committees of the participating institutions in the study with the Declaration of Helsinki.

Informed consent

All the participants included in this study provided written informed consent.

Data availability

All data generated or analysed during this study are included in this published article (and its supplementary information files). Any further information on the datasets generated in the current study can be availed from the corresponding author on request.

Author Contributions

PM and MJ conceived the original idea and planned the experiments. PM carried out the experiments. PM and MJ wrote the manuscript. BKB and SB provided the clinical samples and patient information. HSK provided the support for statistical analysis. NT and HRK helped with miRNA-target network image. US and AJ critically revised the manuscript. MJ supervised and supported the research. All authors contributed to the article and approved the submitted version.

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References


Tables

Table 1 Baseline characteristics of Psoriasis vulgaris (PV) patients and Healthy controls (HC)

<table>
<thead>
<tr>
<th>PV Patients and Healthy controls</th>
<th>Age groups</th>
<th>PASI Score /BSA</th>
<th>No. of patients (n=16)</th>
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<td>PV Patients (n=40)</td>
<td>18-30</td>
<td>Mild (n=5/40)</td>
<td>7</td>
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<tr>
<td></td>
<td>30-40</td>
<td>(PASI &lt;5, BSA&lt;3%)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>Moderate (n=18/40)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>50-60</td>
<td>(PASI 5-10, BSA 3-10%)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>Severe (n=17/40)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(PASI&gt;10, BSA &gt;10%)</td>
<td>3</td>
</tr>
<tr>
<td>Male (28/40)</td>
<td>1</td>
<td>Acanthosis 16</td>
<td>9</td>
</tr>
<tr>
<td>Female (12/40)</td>
<td>1</td>
<td>Parakeratosis 13</td>
<td>1</td>
</tr>
<tr>
<td>Controls(n=40)</td>
<td>1</td>
<td>Hyperkeratosis 13</td>
<td>1</td>
</tr>
<tr>
<td>Male (28/40)</td>
<td>1</td>
<td>Leukocytes infiltration 13</td>
<td>1</td>
</tr>
<tr>
<td>Female (12/40)</td>
<td>1</td>
<td>Elongated rete ridges 12</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Hypogranulosis 12</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2 Differential Gene Expression and Receiver Operating Curve (ROC) analysis with Area under the curve (AUC) for miRNAs expression in plasma samples from Psoriasis vulgaris patients and Healthy controls.

<table>
<thead>
<tr>
<th>Differential expression &amp; ROC Curve Analysis</th>
<th>miR-215</th>
<th>miR-148a</th>
<th>miR-125b</th>
<th>miR-223</th>
<th>miR-142-3p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fold change (p-value)</td>
<td>3.34</td>
<td>2.43</td>
<td>1.75</td>
<td>2.79</td>
<td>2.27</td>
</tr>
<tr>
<td>AUC (p-value)</td>
<td>0.873</td>
<td>0.768</td>
<td>0.709</td>
<td>0.790</td>
<td>0.787</td>
</tr>
<tr>
<td>SE</td>
<td>0.0463</td>
<td>0.0546</td>
<td>0.0617</td>
<td>0.0546</td>
<td>0.0520</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.782 to 0.963</td>
<td>0.661 to 0.875</td>
<td>0.588 to 0.829</td>
<td>0.683 to 0.897</td>
<td>0.685 to 0.889</td>
</tr>
</tbody>
</table>

****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05

Table 3 Pairwise correlation between the miRNAs represented as Pearson correlation coefficient
<table>
<thead>
<tr>
<th>miR-215</th>
<th>miR-148a</th>
<th>miR-125b</th>
<th>miR-142-3p</th>
<th>miR-223</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.19</td>
<td>-0.18</td>
<td>-0.21</td>
<td>-0.32*</td>
</tr>
<tr>
<td>(0.1030)</td>
<td>(0.1297)</td>
<td>(0.0658)</td>
<td>(0.0049)</td>
<td></td>
</tr>
<tr>
<td>miR-148a</td>
<td>1</td>
<td>0.14</td>
<td>0.41*</td>
<td>0.13</td>
</tr>
<tr>
<td>(0.1030)</td>
<td>(0.2131)</td>
<td>(0.0002)</td>
<td>(0.245)</td>
<td></td>
</tr>
<tr>
<td>miR-125b</td>
<td>-0.18</td>
<td>0.14</td>
<td>1</td>
<td>0.25*</td>
</tr>
<tr>
<td>(0.1297)</td>
<td>(0.2131)</td>
<td>(0.6185)</td>
<td>(0.0305)</td>
<td></td>
</tr>
<tr>
<td>miR-142-3p</td>
<td>-0.21</td>
<td>0.41*</td>
<td>0.06</td>
<td>0.32*</td>
</tr>
<tr>
<td>(0.0658)</td>
<td>(0.0002)</td>
<td>(0.6185)</td>
<td>(0.0051)</td>
<td></td>
</tr>
<tr>
<td>miR-223</td>
<td>-0.32*</td>
<td>0.13</td>
<td>0.25*</td>
<td>0.32*</td>
</tr>
<tr>
<td>(0.0049)</td>
<td>(0.2451)</td>
<td>(0.0305)</td>
<td>(0.0051)</td>
<td></td>
</tr>
</tbody>
</table>

*p ≤ 0.05

**Table 4** Select miRNAs with their targets and associated signaling pathways. The targets enlisted for each of the miRNAs represent predicted and/or validated downstream signaling components along with their functional role in psoriatic keratinocytes and/or T cells.
<table>
<thead>
<tr>
<th>miRNAs</th>
<th>miR-215</th>
<th>miR-142-3p</th>
<th>miR-125b</th>
<th>miR-223</th>
<th>miR-148a</th>
<th>Keratinocytes (KC)</th>
<th>T cells</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>KEGG pathways</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WNT signaling pathway</td>
<td>TBL1X, ROCK2, RAC1, PLCB1</td>
<td>ZNRF3, TLE3, DAAM1</td>
<td>SIAH 1, FZD4, APC, PRKACB</td>
<td>SKP1, TBL1XR1, PRICKLE2, PPARD, WNT1, WNT10B, PSENM</td>
<td>Keratinocyte proliferation and apoptosis</td>
<td>T cell development and differentiation</td>
<td>[40, 41]</td>
<td></td>
</tr>
<tr>
<td>MAPK signaling Pathways</td>
<td>TGFBRI, RAC1, IRAK1, MAP4K3, MAP3K11, TAO1, CRK</td>
<td>FGFR2, CACNB1, CACNB3, RASGRF2, RAP1A, FGFR2, MKNK2, SOS2, RPS6KA1, TRAF6, MAP3K11, DUSP7, MAP2K7, DUSP6, MAPK12</td>
<td>IGF1R, RRAS2, ELK4TAOK3MEF2C, TGFA, IGF1, CSF1 KIT, TEK, SOS1, SOS2, NRAS, MRAS, TGFBR2, GADD45A, MAP3K4, DUSP1</td>
<td>Proliferation and differentiation of KCs. miR-125b shown to modulate KC proliferation via FGFR2</td>
<td>T cell activation and differentiation and effector functions</td>
<td>[46-48]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β signaling</td>
<td>ACVR2A, ACVR2B, LEFTY2, ID4, BMPRI2</td>
<td>ACVR2A, TGFBRI</td>
<td>ACVR2B, INHBB, LTBP1, NOG, RGMA, ROCK1, SKP1, TGFBR2, TGFBR2</td>
<td>KC hyperproliferation</td>
<td>T cell homeostasis</td>
<td>[49, 50]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphotidylinositol signaling pathway</td>
<td>PLCB1, IPMK, ITPKB, INPP5A, ITPR3</td>
<td>PI4K2B, MTMR3, CDS2, ITPR3, CALML4, PIK3CA, INPP4A, PTEN</td>
<td>SESN2, PRKAA1, IGF1, SOS1, SOS2NRAS, PIK3R3, PTEN, RICTOR</td>
<td>Regulates hyperproliferation and aberrant differentiation in psoriatic lesions</td>
<td>T cell proliferation and metabolism</td>
<td>[52-54]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mTOR signaling pathway</td>
<td>SLC38A9, GRB10, EIF4EBP1, PRKAA1, DVL3</td>
<td>SESN2, PRKAA1, IGF1, SOS1, SOS2NRAS, PIK3R3, PTEN, RICTOR</td>
<td>SESN2, PRKAA1, IGF1, SOS1, SOS2NRAS, PIK3R3, PTEN, RICTOR</td>
<td>Regulates hyperproliferation and aberrant differentiation in psoriatic lesions</td>
<td>T cell proliferation and metabolism</td>
<td>[55, 56]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFR signaling</td>
<td>DYRKA1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Regulates proliferation and inflammation, miR-215 inhibits proliferation via DYRKA1.</td>
<td>Not studied in context of T cells in Psoriasis</td>
<td>[51]</td>
</tr>
<tr>
<td>KC innervation</td>
<td>Sema3A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Regulates KCs</td>
<td>Not studied in context of T cells in Psoriasis</td>
<td>[59]</td>
</tr>
<tr>
<td>Notch Signaling</td>
<td>BRD4, LFNG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Regulates KC differentiation.</td>
<td>Regulates T cell</td>
<td>[60, 61]</td>
</tr>
<tr>
<td>Pathway</td>
<td>miRNAs</td>
<td>Signaling Effect</td>
<td>References</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>--------</td>
<td>----------------------------------------------------------------------------------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemokine signaling Pathway</td>
<td>DTX4, TLE3, NCOR2, ATXN1</td>
<td>miR-125b regulates cell proliferation via BRD4 in Notch signaling development, activation and effector function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SOS1, SOS2, NRAS, PIK3R3, ROCK1, PREX1</td>
<td>KC induced inflammation miR-125 regulates KC proliferation via STAT3 T cell induced immune-inflammation</td>
<td>[62, 63]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T cell receptor signaling Pathway</td>
<td>MAPK12, MAP2K7, IFN-γ, IL-2RB, IL-10RA, PRDM1</td>
<td>Not relevant in KCs T cell activation and homeostasis.</td>
<td>[30, 64]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-21 signaling</td>
<td>IL-21</td>
<td>Epidermal hyperplasia T cell activation, proliferation and differentiation. miR-215 targets IL-21</td>
<td>[67-69]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-17 signaling</td>
<td>IL-17RA, IL-17RE, RUNX1</td>
<td>Mediator of KC inflammatory loop and hyperproliferation Enhanced Th17 mediated inflammation. miR-215 modulates IL-17 receptors and RunX1.</td>
<td>[70, 72]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell cycle</td>
<td>RB1, CDC7, E2F7, E2F8</td>
<td>Enhanced cell cycle with hyper-proliferation in psoriatic lesions T cell proliferation miR-142-3p targets E2F7, E2F8.</td>
<td>[75, 76]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apoptosis</td>
<td>XIAP</td>
<td>KC survival/apoptosis miR-215 targets XIAP with potential role in T cell apoptosis</td>
<td>[74, 76]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

§ miRNA specific targets shown have been functionally validated as per literature.

**Figures**
Figure 1

Panel A: Representative images of psoriatic patients showing well demarcated scaly, erythematous lesions. Panel B: Representing histopathological features as observed in hematoxylin and eosin-stained sections of lesional and non-lesional tissue from psoriasis vulgaris patients. a. Hyperkeratosis and Acanthosis (black arrow), original magnification x10. b. Extension of rete ridges (black arrow) and leukocytic dermal infiltration (dashed circle with white arrow) in lesional tissue as compared to non-lesional tissue, original magnification x10. c. Reduced granular layer (hypogranulosis) in lesional tissue, original magnification x20.
Figure 2

Panel A

Panel B

Panel C

Figure 2

Panel A: Expression profiling of select miRNAs in plasma samples of Psoriasis vulgaris patients (PV) in comparison to healthy controls (HC): a. miR-215 b. miR-148a c. miR-125b d. miR-223 e. miR-142-3p. Scatter plot represents normalized $2^{-\Delta\Delta C_t}$ values of individual samples. The black dots represent healthy control subjects and PV patients are represented by red dots for miR-215, green dots for miR-148a, blue dots for miR-125b, purple dots for miR-223 and pink dots for miR-142-3p. Data are expressed as mean ± SD. ***p<0.001, **p<0.01 and *p<0.05 calculated using Mann-Whitney U test. The data represent 3 independent qRT-PCR experiments Panel B: Receiver operating characteristics (ROC) curve with area under the curve exhibit the diagnostic value of the select miRNAs a. miR-215 b. miR-148a c. miR-125b d. miR-223 e. miR-142-3p, ****p<0.0001 and ***p <0.001. Panel C: Receiver operating characteristics (ROC) curve exhibit significant diagnostic value of five miRNAs in combination, ****p<0.0001.
Figure 3

a. Pairwise Correlation among the expression levels of the five miRNAs using Pearson correlation. Values shown in the figure depict correlation coefficient ($r$) with significant correlation represented by $^*p \leq 0.05$. b. Correlation analysis of miRNA expression levels with PASI Score using b-spline model. All five miRNAs together exhibit the best fit model with coefficient of determination, $R^2 = 0.94$, Akaike information criterion (AIC) = 131.8. B-spline model for individual miRNAs with PASI score does not exhibit a good fit model.

Figure 4

miRNA-mRNA network mapping for miR-215, miR-148a, miR-223, miR-125b and miR-142-3p as select miRNAs of interest with their direct targets (Please see Table 4). miRNAs are depicted as rectangular boxes and their targets are represented as circles. Color coding of the targets represent their functionality in the
corresponding pathways as highlighted in different colors. Targets in empty circles represent those candidates involved in two or more pathways.

**Fig 5**

Figure 5

Representation of candidate miRNAs involved in keratinocyte and T cell crosstalk dysregulated in psoriasis. The deregulated expression of miR-215, miR-148a, miR-125b, miR-223 and miR-142-3p in keratinocytes and/or T cells is shown to be altered in cell free peripheral circulation

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx