MicroRNA and its link to osteoblasts in adolescent idiopathic scoliosis: abridged secondary publication

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KEY MESSAGES

1. Plasma miR-96 and miR-224 levels significantly increased in those with adolescent idiopathic scoliosis (AIS), compared with controls.
2. Plasma miR-96 and miR-224 correlated with bone quality parameters and bone turnover markers in AIS.
3. Aberrant level of miR-96 and miR-224 may contribute to abnormal osteoblast activities in AIS.

Hong Kong Med J 2020;26(Suppl 7):S10-2
HMRF project number: 04152176

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Introduction

Adolescent idiopathic scoliosis (AIS) is a three-dimensional spinal deformity of unknown cause occurring predominantly in girls aged 10 to 13 years, with a global prevalence of 1% to 4%.[1] Severe curve deformity in AIS is associated with serious functional morbidities, cardiopulmonary compromise, early spinal degenerative changes, and psychosocial disturbance.[2] Bracing is the most recognised non-surgical treatment for skeletally immature patients with the Cobb angle between 25° and 40°, with a success rate of 70% to 75% subjected to good quality, compliance, adequate duration and monitoring.[3] Age, Risser sign, and Cobb angle at initial visit, abnormal bone quality, and menarche have implication for curve progression, but their clinical use remains very limited.[4] To search for more accurate biomarkers for evidence-based treatment of the high-risk group and to avoid unnecessary over-treatment and associated radiation exposure, we aimed to identify circulating biomarkers for diagnosis of AIS. It is speculated that abnormality of AIS bone metabolism results in vulnerable spine. Our previous study revealed a causative relationship between aberrant microRNA-145-5p (miR-145) expression and abnormal osteocyte structure and function associated with low bone mass and qualities in AIS.[5] miRNAs are abundant in bio-fluids and their profiles are relatively stable for long-term clinical studies, thus rendering them as potential biomarkers for human diseases. Serological markers, such as circulating miRNA, might be an alternative indicator reflecting the abnormal bone metabolism in AIS.

This study consisted of two parts: (1) a retrospective case-control study to validate the plasma levels of the miRNA-96, -224, and -605 in AIS and healthy subjects; (2) an in vitro study to elucidate the biological roles of miRNAs in osteoblast osteogenic activities in AIS using a primary osteoblasts culture model.

Methods

Ethical approval was obtained from the Joint Chinese University of Hong Kong – New Territories East Clinical Research Ethics Committee. Written informed consent was obtained from all subjects and their legal guardians. 100 girls with AIS (with Cobb angle evenly distributed from 15° to 80°) were recruited from the Scoliosis Clinic in Prince of Wales Hospital, Hong Kong, and 52 age-matched healthy girls were recruited randomly from local secondary schools as controls. Anthropometrical parameters (standing height, sitting height, body weight, and arm span) were measured with standard protocols. Cobb angle of the major curve was measured within a month before or after blood collection. Bone mineral density and bone qualities were assessed by dual-energy X-ray absorptiometry and high-resolution peripheral quantitative computed tomography, respectively. Serum samples were sent to Chan & Hou Medical Laboratories Ltd (Hong Kong) for assaying level of CTX/P1NP with Elecsys platform (Roche). miRNA level was tested in plasma with real-time PCR.

Iliac crest bone tissues were obtained from patients undergoing posterior instrumentation and spinal fusion requiring iliac crest autografts for AIS with a Cobb angle of >45°. Primary osteoblasts were isolated from bone biopsies. Human osteoblast cell line was used as control. Model of gain or loss of function in miR-96, -224, and -605 was constructed in primary AIS osteoblasts and control osteoblast cell line. mRNA level of representative osteogenic markers was assayed accordingly.

Results

Construction of miRNA signature in AIS

We assayed the expression level of miRNA candidates in plasma of the case-control cohort to construct the miRNA signature of AIS. Levels of miR-96 and miR-224 significantly increased in plasma of AIS, whereas miR-605 was undetectable in both plasm and serum. Area under the curve (AUC) analysis demonstrated discriminating potency of plasma miRNAs between AIS and healthy controls (unpublished data). Therefore, plasma miRNA signature of AIS composed of higher plasma levels of miR-96 and miR-224. Correlation analysis was conducted between the two miRNAs and clinical features in AIS, including Cobb angle, anthropometric information, serological bone metabolic markers, areal bone mineral density (measured with dual-energy X-ray absorptiometry), and bone quality parameters (measured with high-resolution peripheral quantitative computed tomography). Plasma levels of miR-96 and miR-224 in AIS showed correlation to certain bone quality parameters and serological bone turnover markers (CTx and P1NP). This indicate possible relation of plasma miRNA signature to the deranged bone qualities and metabolism in AIS.

Aberrant osteogenic differentiation of primary AIS osteoblasts

Osteoblasts in AIS showed consistently lower (but not significantly) alkaline phosphatase (ALP) activity in a temporal sequence (Fig. 1a). Calcium deposition capacity indicated by Alizarin Red staining showed significantly lower calcium nodules formation at day 14 under osteogenic induction in AIS osteoblast, compared with controls (Fig. 1b & 1c). In addition, AIS osteoblast differentially expressed representative osteogenic markers and exhibited significantly reduced mRNA expression of *Spp1*, *E11*, and *Opg* with osteogenic medium after 7 days culture. The *Rankl/Opg* ratio was consistently higher in AIS osteoblasts (Fig. 1d & 1e).

Regulation of miR-96, -224, -605 in osteogenic activities of osteoblast

Roles of miR-96, -224, -605 on osteoblast activities

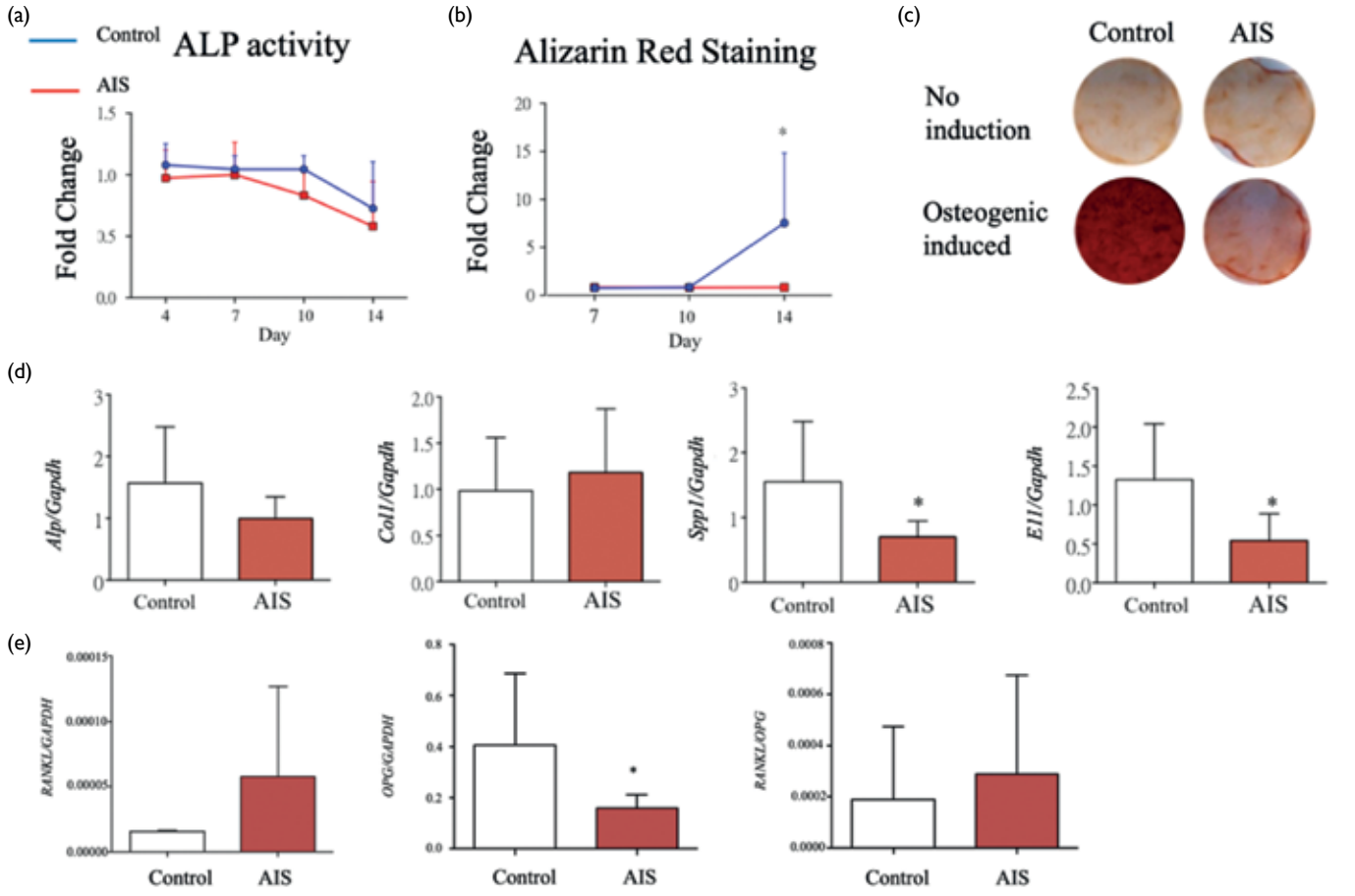

FIG 1. Osteogenic ability of primary osteoblasts in adolescent idiopathic scoliosis (AIS) and healthy controls: semi-quantitative analysis of (a) alkaline phosphatase (ALP) activity and (b) calcium nodules formation (using Alizarin Red staining) of primary osteoblasts from AIS and control cell line under osteogenic induction in temporal sequence. (c) Representative pictures of Alizarin Red staining. (d) mRNA levels of representative osteogenic markers expression of *Alp*, *Col1*, *Spp1*, *E11*. (e) mRNA levels of *Rankl*, *Opg*, and *Rankl/Opg* ratio.

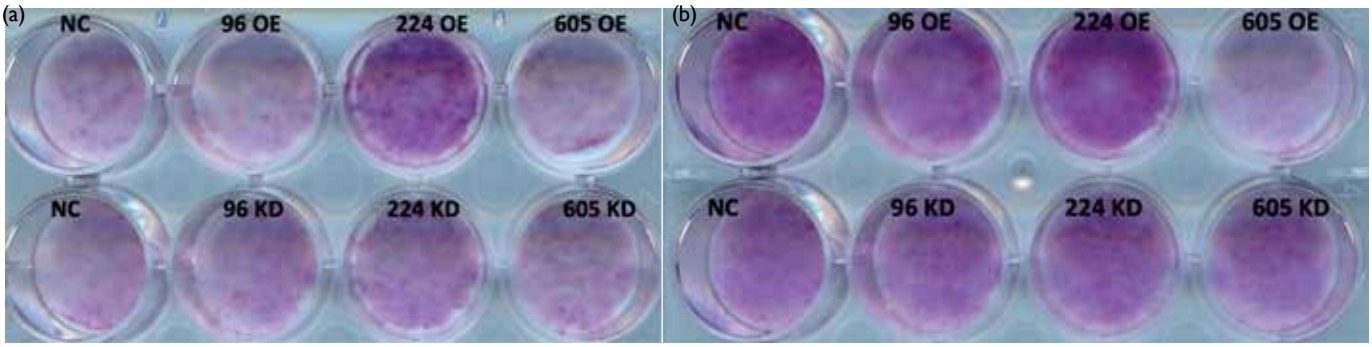

FIG 2. Representative pictures of alkaline phosphatase staining in gain or loss of function in osteoblasts cultured for 5 days in (a) basal medium and (b) osteogenic medium showing overexpression (OE) or knockdown (KD).

were shown in gain- or loss-of-function models in AIS primary osteoblast culture and control osteoblast cell line. Overexpression of miR-224 significantly increased ALP activity in osteoblasts after 5 days culture in basal medium (Fig. 2a). Overexpression of miR-605 significantly reduced ALP activity in osteoblasts after 5 days culture in osteogenic medium (Fig. 2b). mRNA level of representative markers for osteoblast activities were significantly changed in gain- or loss-of-function models.

Discussion

Our study aimed to investigate clinical implication of circulating miRNAs in disease diagnosis by determining associations between bone metabolism and underlying mechanism. Circulating miRNA signature of AIS was constructed from microRNA microarray of bone biopsies from AIS vs non-AIS control, and was validated in the plasma samples of a retrospective case-control cohort. miR-96 and miR-224 had significantly higher levels in AIS plasma and were correlated to the abnormal bone qualities in AIS. Plasma levels of miR-96 and miR-224 had significantly positive correlation with serum levels of P1NP and CTx in AIS. This correlation pattern suggested that miR-96 and miR-224 can be used to reflect bone turnover and pathological alteration of bone cell biology in AIS. In vitro study provided evidence for regulation of miR-96, -224, and -605 in osteogenic activities in osteoblasts with gain- or loss-of-function model. Our study proposed an evidence-based diagnosis of circulating miRNAs to aid decision making in management for AIS. The results enable construction of a model with pathology-associated clinical features and differentially expressed miRNAs. This study is the first step for constructing a reliable predictive system of curve progression at the early presentation of AIS using validated biomarkers and clinical features.

Conclusion

Plasma miRNAs was potential biomarkers to distinguish AIS from healthy controls.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#04152176). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

Disclosure

The results of this research have been previously published in:

1. Zhang J, Chen H, Leung RKK, et al. Aberrant miR-145-5p /β-catenin signal impairs osteocyte function in adolescent idiopathic scoliosis. FASEB J 2018:fj201800281.
2. Chen H, Zhang J, Wang Y, et al. Abnormal lacuna-canalicular network and negative correlation between serum osteocalcin and Cobb angle indicate abnormal osteocytes function in adolescent idiopathic scoliosis. FASEB J 2019;33:13882-13892.
3. Zhang J, Cheuk KY, Xu L, et al. A validated composite model to predict risk of curve progression in adolescent idiopathic scoliosis. EClinicalMedicine 2020;18:100236.

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