UHRF1 Associated with Osteogenic Differentiation of MSCs Contributes to Osteosarcoma Progression and Has Clinical Prognostic Impact in Osteosarcoma

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ABSTRACT: Ubiquitin-like with plant homeodomain and ring finger domains 1 (UHRF1) can mediate DNA methylation and histone modifications in the epigenetic regulation of gene expression, stem cell differentiation and tumorigenesis. Here, we analyzed the differently expressed mRNAs (DEmRNAs) in osteogenesis differentiation of MSCs and osteosarcoma. We identified UHRF1 as the co-DEmRNA to regulate the osteogenesis differentiation of MSCs and osteosarcoma. Moreover, we determined that the functions and pathways of UHRF1 in osteosarcoma. This finding indicates that UHRF1 is closely associated with metastasis and recurrence in osteosarcoma. Based on this finding, we derived a risk signature using UHRF1. In conclusion, UHRF1 plays a crucial role in the malignant progression of osteosarcoma and are potentially useful for osteosarcoma progression treatment strategy development.

KEY WORDS: UHRF1, MSCs, osteogenic differentiation, osteosarcoma, prognostic signature

I. INTRODUCTION

As one of the most common primary malignant tumors, osteosarcoma occurs primarily in children and adolescents. In the treatment, the surgery is still the major method, though the survival of patients with surgery alone is low. In recent decades, surgical treatments and combinational chemotherapy can significantly increase the survival rate. However, metastasis, recurrence, resistance, etc., of tumor seriously affect the prognosis of the patient survival. So, analysis of the potential molecules mechanisms in osteosarcoma development can provide significant biomarkers and treatment methods in the future. Mesenchymal stem cells (MSCs) own the potential capacity to differentiate into osteoblasts, chondrocytes, and adipocytes. In recent years, the function of MSCs in cancer attracted widely. In tumor microenvironment (TME), MSCs can not only anchor tumor cells and secrete tumor growth related factors, but also transdifferentiate into macrophages under the action of cytokines and chemokines, result in tumor growth or inhibition. The pathological differentiation osteosarcoma is closely related to tumor gene mutations. In recent years, there have been reported that activation of related signaling pathways in MSCs can inhibit the development of skeletal anomalies including enchondroma and osteosarcoma. Cytokines and signaling pathway molecules related to osteogenic differentiation of MSCs play an important role in the occurrence and development of osteosarcoma. However, we still do not yet understand the mechanism by which MSCs osteogenic differentiation factors regulate osteosarcoma.

Ubiquitin-like with plant homeodomain and ring finger domains 1 (UHRF1), also called as ICBP90, is a transcription factor regulating the expression of topoisomerase IIα by binding to an inverted CCAAT box located in its promoter. Liu et al. first reported that UHRF1 can promote human osteosarcoma cell invasion by downregulating the expression of E-cadherin via Rb1-dependent manner. However, the literature lacks a comprehensive analysis of the expression of UHRF1 in different clinical features of osteosarcoma, their function in osteosarcoma malignant progression, and their prognostic value.

In this study, we screened databases related to osteogenesis differentiation of MSCs and osteosarcoma, obtaining the co-DEmRNAs. Through analyzing the co-DEmRNAs, we aim to reveal the DEmRNAs.
between osteogenesis differentiation of MSCs and tumor growth in dynamic process, and thus reveal the connection of MSCs between the osteogenesis differentiation and osteosarcoma, offers a new target for the prognosis and treatment of osteosarcoma.

II. MATERIALS AND METHODS

A. Datasets

For the osteogenesis differentiation of MSCs of The Gene Expression Omnibus (GEO) database, RNA-seq data were downloaded from datasets GSE74837 (Platforms: GPL13915 3D-Gene Human Oligo chip 25 k V2.1, Control group: GSM1936199, Osteogenesis differentiation group: GSM1936200 and GSM1936201) and GSE80614 (Platforms: GPL6947, Illumina HumanHT-12 V3.0 expression bead chip, Control group: GSM2131696, GSM2131697, GSM2131698, Osteogenesis differentiation group: GSM2131726, GSM2131727, GSM2131728).

For the osteosarcoma patients of the cancer genome atlas (TCGA) database, tumoral RNA-seq data were downloaded from the genomic data commons (GDC) data portal (TCGA) and 260 of the tumors also had mRNA expression data of paired normal tissue samples. All data of normal tissue samples were obtained from 2 in GTEx V8 release version (https://gtexportal.org/home/datasets). Complete description of the donor genders, multiple ethnicity groups, wide age range, the biospecimen procurement methods and sample fixation were described in GTEx official annotation.

B. Identification and Selection of DEmRNAs

Identification of DEmRNAs was done using GEO2R online analysis (R 3.2.3, Biobase 2.30.0, GEOquery 2.40.0, limma 3.26.8). The Selection of DEmRNAs used Venn analysis from TBtools (Version 1.068). GO function and KEGG analyses were conducted for the DEmRNAs using the database for annotation, visualization and integrated discovery (DAVID) v6.8 (https://david.ncifcrf.gov.).

D. Construction of the UHRF1 Prognostic Signatures

For Kaplan–Meier curves, p values, and hazard ratio (HR) with 95% confidence interval (CI) were generated by log-rank tests and univariate Cox proportional hazards regression. All analytical methods above and R packages were performed using R software version v4.0.3 (the R Foundation for Statistical Computing, 2020), p < 0.05 was considered as statistically significant. Univariate and multivariate cox regression analysis was performed to identify the proper terms to build the forest. The forest was used to show the p value, HR, and 95% CI of each variable through ‘forestplot’ R package.

III. RESULTS

A. Identification of DEmRNAs from Osteogenesis Differentiation of MSCs

Considering the diversity of the DEmRNAs among different datasets about osteogenesis differentiation of MSCs, we chose two datasets to analyze the mRNAs via hierarchical clustering analysis of mRNAs, with p < 0.05 and |logFC| > 1 as the thresholds. We obtained 770 DEmRNAs from GSE74837, including 382 down-regulated DEmRNAs and 388 up-regulated DEmRNAs; and a total of 689 DEmRNAs, including 378 down-regulated DEmRNAs and 311 up-regulated DEmRNAs were obtained from GSE80614 (Fig. 1A to 1D). Concurrently, using Venn diagram to analyze two datasets, we obtained 157 co-DEmRNAs (Fig. 1E).

B. Expression of Co-DEmRNAs is Associated with Differentiation and Growth of MSCs via Regulating Signal Pathways through Extracellular Matrix

To better understand the role of the DEmRNAs in osteogenesis differentiation of MSCs, we analyzed the DEmRNAs by gene ontology (GO) pathway
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Analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (Fig. 2). Through the results from enrichments in GO, we shown that in biological processes (BP), co-DEmRNAs were significantly enriched in signal transduction, cell adhesion, positive regulation of transcription from RNA polymerase II promoter, etc. In cellular component (CC), these co-DEmRNAs were mainly enriched in extracellular space, extracellular exosome, extracellular region, etc. The co-DEmRNAs were also significantly associated with protein and receptor binding in molecular function (MF) (Fig. 2A). KEGG pathway analysis showed that co-DEmRNAs with the highest p value were mainly enriched in Pathways in cancer, TNF signaling pathway, Toll-like receptor (TLR) signaling pathway, etc. (Fig. 2B). In the process of bone regeneration, MSCs and cytokines interact with each other, and promote the process of bone healing. In TNF signaling pathway of MSCs, TNF-α promotes survival and migration of MSCs under oxidative stress via NF-κB pathway. In addition, it has been confirmed that MSCs can release cell chemokines such as chemotactic protein-1 (MCP1) into extracellular matrix, in response to circulating TLR ligands, and induced monocyte into the bloodstream. As demonstrated herein, co-DEmRNAs are mainly regulated signal transduction and molecular expression via secretion pathway of extracellular matrix by mediating membrane related factors to regulate differentiation and growth of MSCs.

C. UHRF1 is Differentially Expressed in Both Osteogenic Differentiation of MSCs and Osteosarcoma

We noticed that these co-DEmRNAs are enriched in tumor-related pathways. So, through screening and analyzing 260 samples of osteosarcoma from TCGA
In our database, we identified 311 up-regulated DEmRNAs and 312 down-regulated DEmRNAs with \( p < 0.05 \) and \(|\log \text{FC}| > 1\) (Fig. 3A and 3B). Using a Venn Diagram to obtain the co-DEmRNAs between osteogenic differentiation of MSCs and osteosarcoma (Fig. 3C), 10 DEmRNAs (IMPA2, KRT19, MCM5, OLFML2B, UHRF1, ALDH2, ALDH4A1, CDC53, FAP, and MMP7) was screened. The KM survival analysis with log-rank test was also used to compare the survival difference with log-rank \( p < 0.05 \) (Fig. 4). Finally, we selected the UHRF1, which is lowly expressed in osteogenic differentiation of MSCs but highly expressed in osteosarcoma. In another study, Stachek et al.\(^7\) confirmed that transcript abundance of UHRF1 decreased in porcine mesenchymal stem cells.

**Fig 2:** GO and KEGG analysis of co-DEmRNAs. The analysis results were based on DAVID bioinformatics (Resources 6.8), and profiled by ggplot2 package in R software (version: 1.3.1093). In the enrichment result, \( p < 0.05 \) is considered to be enriched to a meaningful pathway. (A) Gene ontology (GO) analysis of potential targets of mRNAs. The biological process (BP), cellular component (CC), and molecular function (MF) of potential targets were clustered. (B) The enriched KEGG signaling pathways were selected to demonstrate the primary biological actions of major potential mRNA. The abscissa indicates gene ratio and the enriched pathways were presented in the ordinate.
cells derived from adipose (AD-MSC) and bone marrow tissue (BM-MSC).

D. UHRF1 Regulates Cell Cycle and Proliferation via DNA Replication and Protein Ubiquitination

Further analysis, we annotated the DEmRNAs’ function of osteosarcoma using GO and KEGG analysis (Fig. 5) and showed the GO descriptions including UHRF1 (Table 1). In GO enrichments, we identified two functions of biological process, DNA repair, and protein ubiquitination involved in ubiquitin-dependent protein catabolic process ($p < 0.001$). And in cellular component, UHRF1 was mainly enriched in nuclear chromatin ($p < 0.001$), meanwhile, protein binding ($p < 0.001$) is the molecular function of UHRF1 in osteosarcoma. It has been shown that protein ubiquitination can regulate nuclear transcription to alter tumor proliferation and growth. $^{28–31}$

E. Evident Correlation between the Expression Difference of UHRF1 and OS

Kaplan–Meier and log-rank test were used to determine the relationship between expression difference of UHRF1 and the OS of distinct clinical outcomes and clinicopathological features osteosarcoma patients, with a cut-off threshold of $p < 0.05$ (Fig. 6). The results showed a significant difference in total OS between high and low expression of UHRF1 and prompted that UHRF1 is a risk factor for osteosarcoma ($\log$-rank $p < 0.001$, HR = 2.1, 95% CI = 1.4–3.18, Fig. 6D). In the group with or without radiotherapy, there was no statistical significance of
FIG. 4: Screening UHRF1 from 10 DEmRNAs through KM survival analysis. Kaplan–Meier survival analysis of the gene signature.
the expression of UHRF1 (Kruskal–Wallis test, $p > 0.05$, log-rank $p > 0.05$, Fig. 6A). Similarly, in the radiotherapy and chemotherapy groups, the expression difference of UHRF1 is not statistically significant (Kruskal–Wallis test $p > 0.05$, log-rank $p > 0.05$, Fig. 6B). However, we found that in new tumor types, the expression of UHRF1 is lower in metastasis groups than that in recurrence group though the expression difference in OS of recurrence and metastasis is not statistically significant (Kruskal–Wallis test $p < 0.001$, log-rank $> 0.05$, Fig. 6C).

**FIG. 5:** DEmRNAs’ function of osteosarcoma using GO and KEGG analysis. The analysis results were based on DAVID bioinformatics (Resources 6.8), and profiled by ggplot2 package in R software (version: 1.3.1093). In the enrichment result, $p < 0.05$ is considered to be enriched to a meaningful pathway. (A) Gene ontology (GO) analysis of potential targets of mRNAs. The biological process (BP), cellular component (CC), and molecular function (MF) of potential targets were clustered. (B) The enriched KEGG signaling pathways were selected to demonstrate the primary biological actions of major potential mRNA. The abscissa indicates gene ratio and the enriched pathways were presented in the ordinate.
F. Establishment of Prognosis Model of UHRF1 in Osteosarcoma

We performed univariate and multivariate Cox regression analyses for the TGGA dataset to determine whether the risk signature is an independent prognostic indicator. By univariate analysis, UHRF1 was correlated with the OS, but age, new tumor types and radiation therapy were not correlated with the OS (Fig. 6E). When including these factors into the multivariate Cox regression, UHRF1 is not significantly associated with the OS ($p > 0.05$), meaning that UHRF1 is not an independent risk factor in osteosarcoma (Fig. 6F). These results confirmed that UHRF1 can un-independently predict prognosis in osteosarcoma patients.

IV. DISCUSSION

Osteosarcoma, as a common type of primary bone malignant tumor, arises from malignant mesenchymal cells, producing osteoid or immature bone. Despite various therapies to suppress the progression of osteosarcoma, the 5-year overall survival rate of patients with metastatic or recurrent osteosarcoma remains dismally poor.$^{3,32}$ Therefore, finding a new molecular therapy is urgently required. In recent years, an increasing number of evidence confirmed that MSCs can regulate the factors related to osteosarcoma and release cytokines via the tumor microenvironment, promoting or inhibiting the occurrence and metastasis of tumors.$^{33,34}$ Cancer stem cells (CSCs) belong to tumor cells, owning the principal properties of self-renewal, clonal tumor initiation capacity, and clonal long-term repopulation potential.$^{35}$ Different from normal adult stem cells, CSCs have stronger plasticity and reproductive ability, resulting in treatment of cancer becoming more difficult.$^{36}$ Therefore, the mRNAs expression diversity of osteogenesis differentiation, a differentiation process from adult stem cells to normal tissues, may be compared with the oncogenesis process from CSCs to osteosarcoma.

With the popularity of whole-genome sequencing in recent years, a great number of sequencing data have been uploaded to public databases. Based on the databases, we identified the UHRF1 expression in osteogenic differentiation of MSCs and osteosarcoma. Our data showed that UHRF1 down-regulated expressed in osteogenic differentiation, but overexpressed in osteosarcoma. The GO analysis results confirmed that it existed in nuclear chromatin, binding to protein associated with transcription, and mediating protein degradation through ubiquitination, to regulate DNA repair and cell cycle and promote proliferation of osteosarcoma. These results also confirmed another study about UHRF1 in osteosarcoma.$^{14}$ We further verified the localization of UHRF1 in osteosarcoma and its possible related functions.

Whether the expression level of UHRF1 can be used as a potential prognostic marker is an important topic of research. We confirmed that osteosarcoma

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The prognostic signature derived using UHRF1 was found to be of value, especially in the prognosis of tumor recurrence and metastasis. In addition, we also determined whether there is the prognostic value of the signature for age, new tumor types and radiation therapy in both the CGGA and TCGA datasets. As we observed, though UHRF1 was a significant factor in total OS, it cannot distinguish the OS between tumor recurrence and metastasis, the OS distinction of radiotherapy or not, as well as the OS of radiotherapy and chemotherapy. Through univariate analysis, we determined that UHRF1 is a risk signature in osteosarcoma patients. However, the multivariate COX analysis showed that it cannot be an independently predict prognosis in osteosarcoma patients.

In conclusion, our findings speculated the difference and correlation of mRNAs between osteogenic differentiation of MSCs and osteosarcoma, systematically demonstrated the expression, potential function, and prognostic value of UHRF1 in osteosarcoma. And it also determined that UHRF1 is significantly correlated with the increased expression levels of genes enriched in the biological processes and signaling pathways that promote the development and malignant progression of osteosarcoma. In summary, our study provides important evidence for future examination of the role of UHRF1, and supplies a new biomarker and therapy in the osteosarcoma.

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REFERENCES
