Hyaluronan-CD44 Interaction Promotes KG-1α Cells Proliferation And Migration

Qinpeng Yang [a], Boyan Huang

College of Life science and Technology, Jinan University, Guangzhou 510000, China

[a]Yqinpeng@163.com

Abstract

KG-1α cells, a human acute myeloid leukemia cell line, express a major cell adhesion molecule, CD44, which binds the extracellular matrix component, hyaluronan (HA), at its external domain and interacts with various signaling molecules at its cytoplasmic domain. In this study, we determined that CD44 is the principal HA receptor on AML stem cells. HA-CD44 interaction promotes KG-1α cells proliferation and migration.

Keywords

Hyaluronan, CD44, Proliferation, Migration, KG-1α cells.

1. Introduction

Acute myeloid leukemia (AML) is defined as a clonal proliferation of immature hematopoietic progenitors with varying degree of myeloid differentiation in the bone marrow, peripheralblood, or extra medullary tissues [1]. AML represents the most frequent acute leukemia in adults with a peak of incidence at approximately 65 years, while is more rarely found in children [2]. High-dose chemotherapy is only effective on survival of 30%-40% of AML patients, due to existence subset of malignant cells that are not effectively eliminated by current treatment regiments [3, 4]. Leukemia stem cells (LSCs) theory may explain this failure [5]. The remaining LSCs in patients are in capacity of self-renewal and proliferation, which is the major cause of recurrence after treatment. KG-1α cells, as leukemia stem cell-like cells whose surface maker is CD34 positive and CD38 negative, have self-renewing potential and anti-chemotherapy property. Thus, we choose KG-1α cells as models of AML stem cells and do some correlative studies.

Hyaluronan (HA), which is negatively charged with electricity and a kind of linear non sulfated glycosaminoglycan, is one of the main components of the extracellular matrix (ECM). The structure of HA is mainly composed of repeated fragments of (β-1,4)-D-glucuronic acid and (β-1, 3)-N-acetyl-D-glucosamine. Unlike other glycosaminoglycans, HA does not have core protein, and it is synthesized in the cytoplasmic side of the plasma membrane instead of in endoplasmic reticulum (ER) or Golgi apparatus. HA is excreted into the extracellular matrix after synthesis. On one hand, HA provides structural support for cells with its large molecular network structure. On the other hand, HA regulates multiple cell biological activities, such as growth, migration, differentiation, adhesion and so on [6, 7]. Current studies found that there are four types of receptor of HA in plasma membrane, including CD44, RHAMM (receptor for HA-mediated motility), IVd4 and LEC (liver endothelial cell receptor). Studies have shown that the interaction of HA and CD44 involves in growth and motility of cancer cells and angiogenesis [8, 9].

CD44, a mainly type of receptor non-covalent binding to HA, is a transmembrane-spanning glycoprotein whose molecular weight is varied from 80KDa to 90KDa. The structure and content of CD44 are different from various cells. Generally, the expression of CD44 is high in malignant cell, and it mediates cellular adhesion and accumulation of homogeneous molecule [10]. CD44 gets involved in the formation and metastasis of cancer cells [10], wound healing [11], inflammatory response [12] and lymphocyte homing [13]. The findings showed that the interaction of HA and CD44 directly activated small G protein, Rac1, in mouse epithelial cells, followed by affecting the reorganization of cytoskeleton and changing the direction of cell migration. Other studies proved that

the binding of HA to CD44 or to RHAMM both can activate ERK in vascular endothelial cells, further inducing the expression of some early responsive genes related to cell migration [14]. Therefore, HA/CD44 signaling pathway is very important to cell migration.

2. Materials and methods

2.1 Cell culture

The KG-1α cells were grown in IMDM containing 10% FBS, 100U/ml penicillin, and 100μg/ml streptomycin at 37℃ in a humidified incubator with 5% CO_2.

2.2 Antibodies and other reagents

Anti-CD44/H-CAM monoclonal antibody (Clone:5F12) was purchased from Thermo scientific. Anti-β-actin monoclonal antibody was purchased from Proteintech. Hyaluronan was obtained from Sigma. HitHunter IP_3 FP Assay Kit was obtained from Discoverx.

2.3 Western Blot Analysis

Cellular total protein samples were mixed with sample loading buffer, boiled for 10min, and subjected to SDS-PAGE. Western blot analysis was conducted as described elsewhere [15]. Proteins were quantified by densitometry using WCIF Image J, and the data was normalized to β-actin.

2.4 Measurement of IP3 Production

IP_3 measurement of KG-1α cells was performed in 384well plates using HitHunter IP_3 FP Assay kit as described in Materials and Methods.

2.5 Antibody-blocking experiments

KG-1α cells (the presence or absence of anti-CD44 antibody) were incubated for 30min at 37℃. Then cells were stimulated with 5μl HA solution (200μg/ml) for 1min. Cells were then processed for IP_3 measurement as described above.

2.6 Cell Proliferation assay

KG-1α cells (5×10^3 cells/well) were treated with HA (400μg/ml) or PBS treatment. These cells were then plated in 96-well culture plates in 0.2ml of IMDM containing 10% fetal bovine serum for 96h at 37℃ in 5% CO_2, 95% air. Each group has three parallels. The growth of these cells were analyzed by measuring cell number using the Olympus R1 cell counter.

2.7 Cell Migration assay

4×10^5 cells were suspended in 200μl serum-free IMDM medium and seeded into the upper chamber of each insert. Then, 600μl of IMDM containing 10% FBS (the presence or absence of HA) was added to a 24-well plate. After incubation for 6h at 37℃, the cells that migrated were fixed in a 2% PFA solution and stained for 20min in a 0.1% Crystal Violet solution in PBS.

2.8 Statistics Analysis

The datas were expressed as mean±S.D.

3. Results

3.1 Expression of CD44 in KG-1α cells

By western blotting, we detected the expression of CD44 in hematopoietic stem cells (HSCs) and KG-1α cells. We found that CD44 strongly expressed in KG-1α cells, which did not express in HSCs.

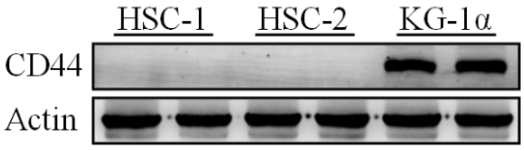

Fig. 1 Western blot analysis of CD44 expression in HSCs and KG-1α cells.

3.2 CD44 is the principal HA receptor on KG-1α cells

CD44 highly expresses in many human tumor cells. We hope to determine which one of HA receptors is particularly important to the capacity of KG-1α cells to interact with HA. To address this issue, we performed antibody-blocking assays using anti-CD44 antibody. Here, we determined that IP_3 production occurs immediately rise in KG-1α cells without anti-CD44 antibody treated. While Pre-incubation with anti-CD44 antibody, IP_3 production was reduced in KG-1α cells treated with HA. The results indicated that CD44 is the principal HA receptor and HA induces IP_3 production in a CD44-dependent manner in KG-1α cells.

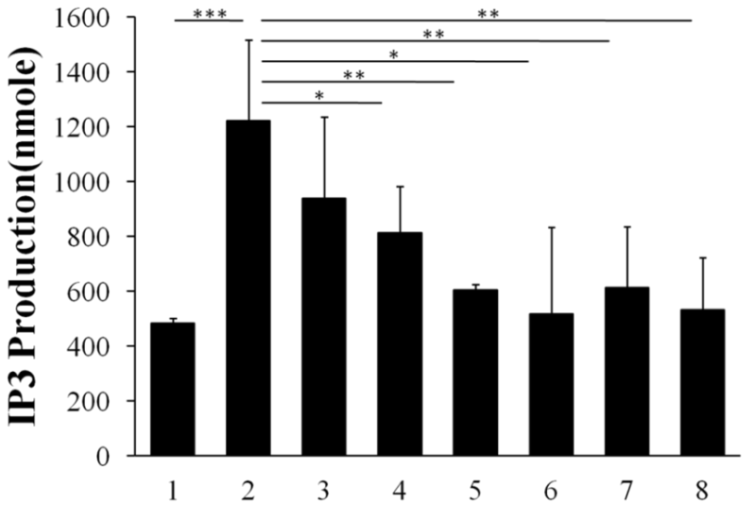

Fig.3.2 Measurement of IP3 Production in KG-1α cells. 1: Cells had no HA treatment. 2: Cells were treated with HA. 3-8: Cells were pretreated with anti-CD44 antibody (10, 30, 50, 55, 60 and 65μg/ml) followed by HA treatment. (n=3; *P<0.05, **P<0.01, ***P<0.001)

3.3 HA-CD44 interaction promotes KG-1α cells proliferation

In an attempt to identify the effect of HA on KG-1α cells proliferation, we treated KG-1α cells with HA (400μg/ml) and couned by the cell counter. We found that the proliferation rate of KG-1α cells treated with HA was significantly higher than the control group. Therefore, HA promotes the proliferation of KG-1α cells.

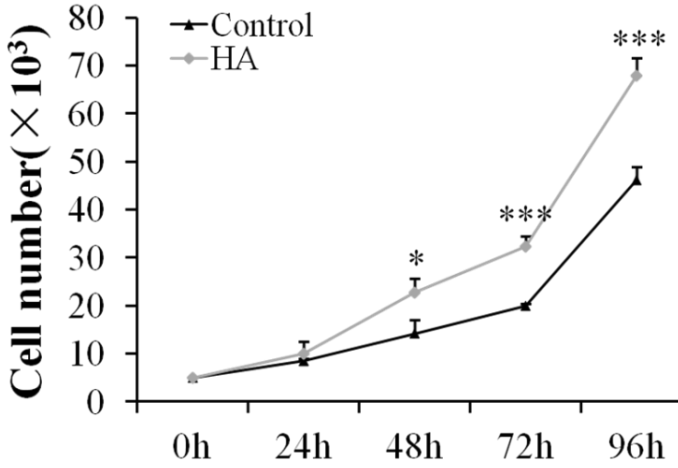

Fig.3.3 The effect of hyaluronan on KG-1α cells proliferation. (n=3; *P<0.05, ***P<0.001)

3.4 HA-CD44 interaction promotes KG-1α cells migmation

KG-1α cells treated with PBS (as control) or HA were respectively seeded on the membrane of transwell insert, the cells passed through the membrane was imaged after 6h. The results showed that the number of cell migration induced by HA was significantly increased, compared with the control

cells. Therefore, these observations demonstrated that HA-CD44 interaction promotes the migration of KG-1α cells.

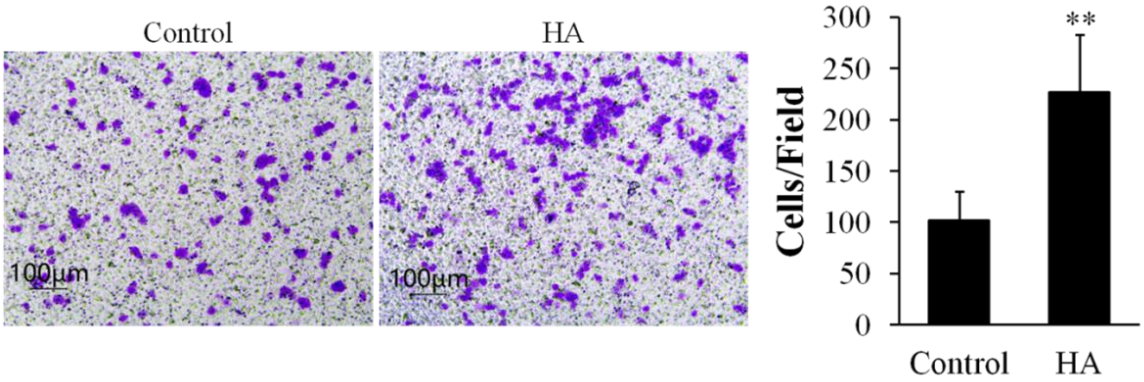

Fig.3.4 The effect of hyaluronan on KG-1α cells migration. (n=5; **P<0.01)

4. Conclusion

In this research, we found that CD44 is the main receptor of HA in KG-1α cells. Low expression or no expression of CD44 in HSCs and strong expression in LSCs implied that we could modify HA by some technical methods, allowing HA carrying anti-cancerogen to target LSCs and achieving the goal of killing the leukemia cells. HA-CD44 interaction improves the production of IP_3, which is the second messenger in KG-1α cells. Phospholipase C (PLC) is capable of hydrolysis of PIP2 to produce IP_3 and DAG. IP_3 binds to its receptor on ER and open Ca^{2+} channel, resulting in releasing Ca^{2+} into the cytosolic, followed by regulating cell life activities, such as proliferation, migration, adhesion and so on [16]. In this study, we also observed that HA-CD44 interaction promoted KG-1α cells proliferation and migration. Thus, we speculated that Ca^{2+} regulation is the reason for AML-like stem cells proliferation and migration induced by HA.

References

[1] Vardiman J W, Thiele J, Arber D A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes[J]. Blood, 2009, 114(5): 937-951.
[2] Deschler B, Lübbert M. Acute myeloid leukemia: epidemiology and etiology[J]. Cancer, 2006, 107(9): 2099-2107.
[3] Roboz G J, Guzman M. Acute myeloid leukemia stem cells: seek and destroy[J]. Expert review of hematology, 2009, 2(6): 663-672.
[4] Walter R B, Appelbaum F R, Tallman M S, et al. Shortcomings in the clinical evaluation of new drugs: acute myeloid leukemia as paradigm[J]. Blood, 2010, 116(14): 2420-2428.
[5] Bonnet D, Dick J E. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell[J]. Nat Med, 1997, 3(7): 730-737.
[6] Toole B P. Hyaluronan: From extracellular glue to pericellular cue[J]. Nat Rev Cancer, 2004, 4(7): 528-539.
[7] Heldin P. Importance of hyaluronan biosynthesis and degradation in cell differentiation and tumor formation[J]. Braz J Med Biol Res, 2003, 36(8): 967-973.
[8] Wang S J, Bourguignon L Y. Hyaluronan-cd44 promotes phospholipase c-mediated ca2+ signaling and cisplatin resistance in head and neck cancer[J]. Arch Otolaryngol Head Neck Surg, 2006, 132(1): 19-24.
[9] Delpech B, Girard N, Bertrand P, et al. Hyaluronan: Fundamental principles and applications in cancer[J]. J Intern Med, 1997, 242(1): 41-48.
[10] Gadhoum Z, Delaunay J, Maquarre E, et al. The effect of anti-cd44 monoclonal antibodies on differentiation and proliferation of human acute myeloid leukemia cells[J]. Leuk Lymphoma, 2004, 45(8): 1501-1510.

[11] Kaya G, Rodriguez I, Jorcano J L, et al. Selective suppression of cd44 in keratinocytes of mice bearing an antisense cd44 transgene driven by a tissue-specific promoter disrupts hyaluronate metabolism in the skin and impairs keratinocyte proliferation[J]. Genes Dev, 1997, 11(8): 996-1007.
[12] Gunthert U. Importance of cd44 variant isoforms in mouse models for inflammatory bowel disease[J]. Curr Top Microbiol Immunol, 1999, 246: 307-312; discussion 313.
[13] Siegelman M H, DeGrendele H C, Estess P. Activation and interaction of cd44 and hyaluronan in immunological systems[J]. J Leukoc Biol, 1999, 66(2): 315-321.
[14] Schmitz A A, Govek E E, Bottner B, et al. Rho gtpases: Signaling, migration, and invasion[J]. Exp Cell Res, 2000, 261(1): 1-12.
[15] Yan D, Mayranpaa M I, Wong J, et al. Osbp-related protein 8 (orp8) suppresses abca1 expression and cholesterol efflux from macrophages[J]. J Biol Chem, 2008, 283(1): 332-340.
[16] Rhee S G. Regulation of phosphoinositide-specific phospholipase c[J]. Annu Rev Biochem, 2001, 70: 281-312.