Upregulation of ADAM12 contributes to accelerated cell proliferation and cell adhesion-mediated drug resistance (CAM-DR) in Non-Hodgkin’s Lymphoma

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ABSTRACT
Objective: ADAM12 is a member of a disintegrin and metalloproteinase family and has been reported to participate in the development of variety of tumors. However, the role of ADAM12 in Non-Hodgkin Lymphoma (NHL) has not been investigated. The present study was undertaken to determine the expression and biologic function of ADAM12 in human NHL.

Methods: First, we constructed a model of cell adhesion in NHL, the mRNA, and protein level of ADAM12 in suspension and the adhesion model was analyzed by RT-PCR and western blot. Then, flow cytometry assay and western blot were used to investigate the mechanism of ADAM12 in the proliferation of NHL cells. In vitro, after using siRNA interfering ADAM12 expression, we performed adhesion assay and cell viability assay to determine the effect of ADAM12 on adhesive rate and drug sensitivity.

Results: ADAM12 was lowly expressed in suspended cells and highly expressed in adherent NHL cells. In addition, ADAM12 was positively correlated with the proliferation and apoptosis of NHL cells by regulating the expression of p-AKT and p-GSK-3β. Furthermore, ADAM12 promoted cell adhesion-mediated drug resistance (CAM-DR) in DLBCL via AKT signaling pathway.

Conclusion and discussion: Our data support a role for ADAM12 in NHL cell proliferation, adhesion, and drug resistance, and it may pave the way for a novel therapeutic approach for CAM-DR in NHL.

KEYWORDS
ADAM12; AKT/PI3K signaling pathway; CAM-DR; cell proliferation NHL

Introduction
Non-Hodgkin’s Lymphoma (NHL) is the most common hematologic malignancy and it is the 6th most common type of cancer in both males and females accounting for 5 and 4% of new cancer cases, respectively [1]. Despite intensive efforts in developing a clinical challenge, effective treatments to achieve a long-term disease control, there is still no consensus over how the optional therapy should deal with the refractory patients [2]. Various therapeutic measures are currently available for refractory NHL patients. However, because of the poor responses from refractory patients, their treatment represents an unmet medical need, requiring novel treatments.

The microenvironment in NHL is composed of a large variety of inflammatory and stromal cells, such as OCI-Ly1, OCI-Ly3, OCI-Ly8, OCI-Ly10 cells, and Raji cells. There is substantial variability in the composition of microenvironment [3]. The PI3K/AKT/mTOR pathway plays an important role on NHL growth and survival, data suggest that PI3Kδ inhibition can overcome prosurvival signals from the microenvironment that maintain NHL cells [4]. Lymphoma cells adhered to stromal cells are important in mediating tumor resistance to cytotoxic therapy (cell adhesion-mediated drug resistance (CAM-DR)) [5]. Previous studies have shown that the human bone marrow stromal cell adhered to B-cell lymphoma cells can protect NHL cells from rituximab cytotoxicity [6]. However, the role of the molecular mechanisms in mediating the resistance of NHL cells to rituximab has not been extensively studied.

ADAM12 is a member of the ADAM family of proteins, a MMP-related metalloproteinase family. ADAM12 is a kind of multifunctional protein; it participates in the proteolytic processing of other transmembrane proteins including cell adhesion, transcription, cell signaling events, RNA metabolism, apoptosis, and cell cycle progression [7,8]. We previously reported that overexpression of ADAM12 in NHL cells using DNA microarray analysis indicated ADAM12 could be an important target molecule for tumor therapy [9]. A lot of reports showed that the expression of ADAM12 was upregulated in many tumors, such as oral squamous cell carcinoma, lung, kidney, brain, breast, skin, and liver tumors, with a close relationship to parameters of cancer development or prognosis [7,10,11]. But, the expression of ADAM12 is also down-regulated in some tumors. Yet, whether or not
ADAM12 has any clinical significance in NHL remains uncertain.

In this study, we explored whether the expression level of ADAM12 and its associated molecular mechanisms participated in NHL’s CAM-DR. We proposed that ADAM12 played an important role in CAM-DR processes through AKT signaling pathway. Our results suggested a creative role of ADAM12 in NHL’s cell proliferation, cell’s apoptosis, and a novel therapeutic method of NHL.

Materials and methods

Patients and cell lines

The resected specimens were obtained from the Department of Pathology, Affiliated Cancer Hospital of Nantong University (Nantong, China). We chose the normal CD19-purified B-cells and DLBCL patients’ cell lines including OCI-Ly1, OCI-Ly3, OCI-Ly8, and OCI-Ly10 cells. Institutional approval was required from Nantong University prior to this study.

mRNA expression analysis using real-time quantitative reverse transcriptase-polymerase chain reaction

Real-time reverse transcriptase-polymerase chain reaction was performed to examine mRNA expression of ADAM12 in normal CD19-purified B-cells and DLBCL cell lines. According to the manufacturer’s instructions, the widespread use of qRT-assays for the diagnosis and detection of disease-specific prognostic markers for patients was provided [12]. ADAM12 primers were: forward primer 5′-ATCAGTGCTTCGGCGTTCA-3′ and reverse primer 5′-GGCAATTCTTCCTGTTACATACC-3′. After amplification, the products were separated on agarose gel and visualized under UV light.

Cell culture

Murine OCI-Ly1, OCI-Ly3, OCI-Ly8, OCI-Ly10, and Raji cells were cultured in RPMI1640 containing 10% fetal bovine serum (FBS). Monolayer cultures were maintained at 37°C in a 5% (v/v) CO2 atmosphere. For all experiments, serum-free conditions used an identical medium without FBS.

Western blot analysis

Western blot was carried out as previously described [13]. According to the manufacturer’s instructions, the antibodies used in this study included: anti-ADMA12 antibody and anti-β-actin antibody (1:1000, Santa Cruz Biotechnology, CA, U.S.A.), AKT, GSK-3β, p-AKT, and p-GSK-3β (Cell Signaling Technology) values were responsible for at least three independent experiments.

Preparation of siRNA and transient transfection

Downregulation of ADAM12 in NHL cells came from siRNA-ADAM12 and control-siRNA as a loading control. Full-length ADAM12 cDNA was obtained from a Non-Hodgkin’s Lymphoma library using PCR, and transfected into pcDNA3.1 construct. We used the Lipofectamine 2000 of transfection reagent to transf ect according to the manufacturers’ instructions.

Soft agar colony analysis

The soft agar colony formation assay is a method used to confirm cellular anchorage-independent growth in vitro. The goal of this protocol is to illustrate a stringent method for the detection of the tumorigenic potential for transformed cells and the tumor suppressive effects of proteins on transformed cells. NHL cells were re-suspended at 2 × 10^3 cells in 1 ml of 0.70% agar solution including cell culture medium and layered on top of a 0.8% agar layer in 24-well plates. Plates were then kept for 2 weeks at 37°C with 5% CO2.

Cell viability assays

Cell viability reagent functions as a cell health indicator by using the reducing power of living cells to quantitatively measure the proliferation of various human and animal cell lines, bacteria, plant, and fungi allowing us to establish relative cytotoxicity of agents within the cytosol of the cell [14]. CCK-8 reagents were added to the every subset well. The absorbance of the cells was quantitated in a microplate reader at 450 nm with a reference wavelength of 630 nm.

Cell cycle assays

After cells were collected, they were fixed in 70% ethanol at −20°C overnight and then incubated with 1 mg/ml RNase A for 30 minutes at 37°C. Subsequently, cells were stained with propidium iodide (50 mg/ml PI; Becton–Dickinson, San Jose, CA, U.S.A.) in PBS with 0.5% Tween-20 in the dark, and analyzed using a Becton–Dickinson flow cytometer BD FACScan.

Cell apoptosis assays

Drug-induced apoptosis, following exposure to Doxo (Sigma-Aldrich, Rehovot, Israel), was detected in NHL cells. In brief, following 48 hours of chemotherapy agent exposure, apoptotic cells were detected using Annexin-V-FLUOS Staining Kit (Roche, Basel, Switzerland) according to the manufacturer’s protocol. Cells were washed three times and re-suspended in 100 l
of AnnexinV-FLUOS labeling solution at a concentration of $1 \times 10^6$ cells/ml incubated in the dark for 15 minutes. The labeled cells were analyzed via flow cytometry (BD FACSARiall).

**Statistical analysis**

The result of expression of ADAM12 was analyzed by SigmaPlot and shown as mean plus or minus standard deviation or standard error regarding the mean of at least three experiments. The results are presented as the means ± SEM. Statistical analysis was conducted using the Statistical Analysis Software package and $p$-value of <0.05 was deemed statistically significant.

**Results**

**ADAM12 is highly expressed in DLBCL b-cells and cell lines**

We investigated the alterations of ADAM12 protein in NHL cell lines. According to the previous research, ADAM12 is related to the development of cancers including head and neck cancer, lung cancer, and hepatocellular carcinoma. In our research, we explored the expression of ADAM12 in DLBCL cell lines (OCI-Ly1, OCI-Ly3, OCI-Ly8, and OCI-Ly10) and normal patients’ cells (four normal CD19 purified B-cells from healthy donors) by qPCR. The experimental results showed that ADAM12 was highly expressed in DLBCL lines compared with healthy men (Figure 1(a)). Further from the perspective of protein level, we examined 12 DLBCL patients’ B-cells and DLBCL cell lines and four normal patients’ cells using western blot. It indicated that ADAM12 was also highly expressed in DLBCL B-cell and cell lines; however, in healthy cells, the expression of ADAM12 did not change significantly (Figure 1(b,c)).

**Downregulation of ADAM12 by siRNA is associated with decreased expression of cell cycle-associated protein p27**

To examine whether ADAM12 was required for the cell cycle progression, OCI-Ly8 and Raji cells were transiently transfected with ADAM12-siRNA or control-siRNA. After transfection for 48 hours, we examined the efficiencies of ADAM12-siRNA-mediated

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**Figure 1.** ADAM12 is highly expressed in DLBCL B-cells and cell lines. (a) ADAM12 is expressed in normal B-cells and DLBCL cell lines through using qPCR. CT value of $\beta$-actin was subtracted from the CT value of ADMA12. (b) We examined the expression of ADAM12 in normal B-cells and DLBCL cell lines using western blot. (c) The histogram demonstrated the ratio of ADAM12 protein level to $\beta$-actin. Data were the mean ± SEM of three independent experiments.
downregulation by western blot (Figure 2(a)). We identified that the efficiency of ADAM12-siRNA#4 was significantly reduced, so the following experiment would use the ADAM12-siRNA#4. The expression levels of P27, Integrin β1 in cells transfected with ADAM12-siRNA, were significantly lower than those of control-siRNA (Figure 2(b)). Experimental results showed that the level expression of ADAM12 was obviously associated with cell cycle progression. Alternatively, flow cytometric analysis indicated that the percentages of cells in G0/G1 phase significantly increased when cells were transfected with ADAM12-siRNA#4 but reduced percentages of cells in S phase; this further proved that the expression of ADAM12 was associated with cell cycle progression (Figure 2(c)).

**ADAM12 promoted the proliferation of NHL cells**

There are many reports indicating that members of ADAM family are highly expressed in human tumors [7]. We supposed that the expression of ADAM12 was associated with tumor cell proliferation. OCI-Ly8 and Raji cells were, respectively, transfected with ADAM12-siRNA#4 and control-siRNA, and after 48 hours, western blot assay was performed to examine the efficiency of transfection. Meanwhile, we used the proliferative marker PCNA as a reference standard. From the experimental results, it was obvious that the expression of ADMA12 significantly decreased 96 hours after transfection, and the expression of PCNA also decreased when using knockdown ADAM12 (Figure 3(a)). Then, to further study that ADMA12 was involved in cell proliferation, we examined the cell growth rate and relative colony formation by CCK-8 assays and soft agar colony assays when using siRNA knockdown ADAM12. Similarly, the data indicated that the downregulation of ADAM12 inhibited the NHL cells’ growth rate and colony formation (Figure 3(b,c)). Furthermore, pcDNA3.1 tagged ADMA12 (Flag-ADMA12) was transfected into OCI-Ly8 and Raji cells. Western blot assay was performed to examine the efficiency of transfection. Results indicated that overexpression of ADAM12 resulted in significant increase of PCNA expression (Figure 3(d)). CCK-8 assays proved that overexpression of ADAM12 accelerated the cell growth rate (Figure 3(e)). In order to further confirm the role of ADAM12 in NHL cells’ proliferation, a pBabe retrovirus system was prepared to intend ADMA12-stable-expressing OCI-Ly8 and Raji cells. We selected the puromycin with virus infection cells, and performed western blot assay to examine the expression of tagged ADAM12. Colony formation

![Figure 2](image-url)

**Figure 2.** Downregulation of ADAM12 by siRNA is associated with decreased expression of cell cycle marker P27. (a) OCI-Ly8 and Raji cells were transfected with ADAM12-siRNA or control-siRNA. Efficiency of ADAM12-siRNA-mediated downregulation was confirmed by western blotting using β-actin as the loading control. (b) The levels of expression of P27, integrin β1 in cells transfected with ADAM12-siRNA, or control-siRNA were analyzed by western blot, and tubulin was a loading control. (c) OCI-Ly8 and Raji cells were transfected with control-siRNA or ADAM12-siRNA and flow cytometric analysis was performed to examine cell cycle progression.
assay indicated that ADAM12 significantly accelerated the number of colonies (Figure 3(f)). All these signs proved that ADAM12 played a profound role in NHL cell proliferation.

ADAM12 regulated cell proliferation and apoptosis via the expression of p-AKT and p-GSK-3β

It previously demonstrated that the expression of ADAM12 was associated with breast cancer proliferation and the regulation of the AKT signaling pathway [15]. Therefore, we next researched whether the expression of ADAM12 was associated with NHL cells’ proliferation through AKT signaling pathway. In order to check this hypothesis, we used siRNA to downregulate ADAM12 in OCI-Ly8 and Raji cells, at the same time, using control-siRNA as a contrast. Western blot assays were performed to examine the overexpression of ADAM12. At the same time, PCNA was also examined. (e) Flag-ADAM12 and Flag-control were used to analyze the cell growth rate by CCK-8 assays. (f) Soft agar assays indicated that ADAM12 dramatically increased colony numbers. The data were means ± SEM of three independent experiments *, #, P < 0.05.
with high activity) was selected to examine the function of AKT regarding the proliferative role of ADAM12. KRX-0401 dramatically downregulated the expression of p-AKT and p-GSK-3β in OCI-Ly8 and Raji cells, respectively (Figure 4(c)). Subsequently, after OCI-Ly8 and Raji cells were treated with AKT inhibitor, CCK-8 assay indicated that Flag-ADAM12-mediated cell viability was significantly reduced by KRX-0401 in OCI-Ly8 and Raji cells (Figure 5(d)). Flow cytometric analysis also indicated the role of ADAM12 in apoptosis. Experimental results showed when ADAM12-siRNA#4 transfected into OCI-Ly8 or Raji cells, the percentage of apoptotic cells significantly increased compared with control-siRNA. Moreover, the effect of ADAM12-siRNA-mediated cell apoptosis was more remarkable when interfered with KRX-0401 and vice versa with overexpression of ADAM12 (Figure 4(e)). These data suggested that ADAM12 regulated cell proliferation and apoptosis via the expression of p-AKT and p-GSK-3β.

**Figure 4.** ADAM12 regulated cell proliferation and apoptosis via the expression of p-AKT and p-GSK-3β. (a) OCI-Ly8 and Raji cells were transfected with control-siRNA or ADAM12-siRNA#4, and western blot assays were performed to examine the expression of AKT, p-AKT, GSK-3β, and p-GSK-3β. (b) Flag-control and Flag-ADAM12 were transfected into OCI-Ly8 and Raji cells, and western blot was used to analyze AKT, p-AKT, GSK-3β, and p-GSK-3β. (c) Treatment of OCI-Ly8 and Raji cells with AKT inhibitor KRX-0401 or DMSO, using western blot to examine the expression of AKT, p-AKT, GSK-3β, and p-GSK-3β. (d) Flag-control or Flag-ADAM12 was transfected into OCI-Ly8 and Raji cells, then, cells with KRX-0401 or DMSO were treated, and CCK-8 assay was performed to examine cell viability. (e) control-siRNA, ADAM12-siRNA#4, Flag-control, Flag-ADAM12 respectively were transfected into OCI-Ly8 and Raji cells, then cells with KRX-0401 or DMSO were treated and flow cytometric analysis was used to examine apoptosis. *, * P < 0.05.
To research whether ADMA12 was associated with CAM-DR, we used NHL cells adhered to fibronectin (FN) or cultured in suspension (sus). Western blot assay was performed to examine the expression of ADMA12 in two different states. Results showed that the expression of ADAM12 significantly increased in adhesion state (Figure 5(a)). Doxorubicin is recognized as a target for the treatment of NHL. We chose NHL cells, OCI-Ly1, OCI-Ly8, and OCI-Ly10 cells for the subsequent assays. CCK-8 assay indicated that downregulation of ADAM12 only slightly decreased cell viability in both the adhesion and suspension states. But, when OCI-Ly1, OCI-Ly8, and OCI-Ly10 treated with Doxorubicin, the cells with adhesion dramatically protected all the three cell lines from Doxorubicin compared with the cells cultured in suspension. Generally, the siRNA-ADAM12 could significantly decrease cell viability in adhesion state (Figure 5(b)). All data indicated that ADAM12 played a significant role in CAM-DR. To research whether AKT signaling pathway was associated with ADAM12-mediated CAM-DR, OCI-Ly1, OCI-Ly8, and OCI-Ly10 cells were treated with KRX-0401 or DMSO. Overexpression of ADAM12 reversed CAM-DR with KRX-0401 treatment of cells through CCK-8 assay (Figure 5(c)). All these discoveries showed that ADAM12 promoted CAM-DR in DLBCL via AKT signaling pathway.

Discussion

A number of studies have demonstrated a critical role of ADAM12 in various solid tumors. But, little research has been done on blood tumor, such as Non-Hodgkin’s Lymphoma. In head and neck cancer, the ADAM12 (a
disintegrin and metalloproteinases) has been described and subsequently found to be increased and increase cancer cells' migration and invasion. In human glioblastomas, ADAM12 is associated with cell proliferation and a shedding of heparin-binding epidermal growth factor. In breast tumor, ADAM12 played an important role in cell progression and cell apoptosis. In gastric cancer, ADAM12, ADAM9, ADAM15 were upregulated. In lung adenocarcinoma, ADAM12 is a prognostic factor [16–20].

However, the function of ADAM12 in Non-Hodgkin’s Lymphoma development is not clear. In our research, we demonstrated that the expression of ADAM12 was associated with the patients with NHL, and overexpression of ADAM12 could promote the cells; proliferation; moreover, downregulation of ADAM12 also could be associated with cell cycle.

A large number of previous studies have demonstrated that tumor microenvironments affected the tumor development, differentiation, migration and response to chemotherapy in NHL, breast cancer, head and neck cancer [21,22]. In order to resist the traditional chemotherapy, we had to be necessary to figure out the molecular mechanisms of CAM-DR, it may be a successful method to alleviate the symptoms of NHL. Lafuste et al. [23] found that ADAM12 was instrumental in human myogenic cell differentiation and associated with the phosphorylation of AKT. Rao et al. [18] demonstrated that PI3K/AKT/mTOR pathways could regulate the expression of ADAM12. In skeletal myoblasts, the opposing effects of ADAM12-siRNA and lack of P27 expression and P130 could inhibit cell proliferation and induce the inactivation of PI3K/AKT signaling pathway [24]. Leyme et al. [25] reported that ADAM12 could regulate cell survival through a PI3K/AKT signaling pathway and mediate the functional association with ILK with β1 integrin. This data reminded us to examine whether ADAM12 could regulate cell survival and CAM-DR in NHL through AKT/PI3K signaling pathway. Prior to the present research, many characterizations of ADAM12 could promote cell proliferation and inhibit cell apoptosis via the expression of p-AKT and p-GSK-3β. Dramatically, ADAM12 regulated CAM-DR through PI3K/AKT signaling pathway. But, the specific mechanisms of ADAM12 in mediating drug sensitivity of NHL need further research.

In summary, our findings support that ADMA12 played an important role in cell proliferation and cell apoptosis, and PI3K/AKT pathway played a crucial role in CAM-DR in NHL. According to our findings, ADAM12 would perform as a crucial sensor in the regulation of the biological function of tumor cells, and the equilibrium was set-up between cell signaling pathway and rational use of cancer drug. Furthermore, this method may be an ideal and effective for the treatment of NHL.

Disclosure statement
No potential conflict of interest was reported by the authors.

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References
[1] Grover NS, Park SL. Novel targeted agents in Hodgkin and Non-Hodgkin Lymphoma therapy. Pharmaceuticals. 2015;8(3):607–636.
[2] Carlo-Stella C, Santoro A. Microenvironment-related biomarkers and novel targets in classical Hodgkin’s lymphoma. Biomark Med. 2015;9(8):807–817.
[3] Wein F, Kappers R. The role of T cells in the microenvironment of Hodgkin lymphoma. J Leukoc Biol. 2016 Jan;99(1):45–50.
[4] Meadows SA, Vega F, Kashishian A, et al. PI3Kdelta inhibitor, GS-1101 (CAL-101), attenuates pathway signaling, induces apoptosis, and overcomes signals from the microenvironment in cellular models of Hodgkin lymphoma. Blood. 2012;119(8):1897–1900.
[5] Miao X, Xu X, Wu Y, et al. Overexpression of TRIP6 promotes tumor proliferation and reverses cell adhesion-mediated drug resistance (CAM-DR) via regulating nuclear p27(Kip1) expression in non-Hodgkin’s lymphoma. Tumour Biol: J Int Soc Onco developmental Biol Medi. 2016;37(1):1369–1378.
[6] Mraz M, Zent CS, Church AK, et al. Bone marrow stromal cells protect lymphoma B-cells from rituximab-induced apoptosis and targeting integrin alpha-4-beta-1 (VLA-4) with natalizumab can overcome this resistance. Br J Haematol. 2011;155(1):53–64.
[7] Mochizuki S, Okada Y. ADAMs in cancer cell proliferation and progression. Cancer Sci. 2007;98(5):621–628.
[8] Seals DF, Courtneidge SA. The ADAMs family of metalloproteases: multidomain proteins with multiple functions. Genes Dev. 2003 Jan 1;17(1):7–30.
[9] Uehara E, Shibata M, Shinozuka K, et al. Upregulated expression of ADAM12 is associated with progression of oral squamous cell carcinoma. Int J Oncol. 2012;40(5):1414–1422.
[10] Murphy G. The ADAMs: signalling scissors in the tumour microenvironment. Nat Rev Cancer. 2008;8(12):929–941.
Kveiborg M, Albrechtsen R, Couchman JR, et al. Cellular roles of ADAM12 in health and disease. Int J Biochem Cell Biol. 2008;40(9):1685–1702.

Kroh EM, Parkin RK, Mitchell PS, et al. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods. 2010;50(4):298–301.

Hang Q, Fei M, Hou S, et al. Expression of Spy1 protein in human non-Hodgkin’s lymphomas is correlated with phosphorylation of p27 Kip1 on Thr187 and cell proliferation. Med Oncol. 2012;29(5):3504–3514.

Marks DC, Belov L, Davey MW, et al. The MTT cell viability assay for cytotoxicity testing in multidrug-resistant human leukemic cells. Leuk Res. 1992;16(12):1165–1173.

Atfi A, Dumont E, Colland F, et al. The disintegrin and metalloproteinase ADAM12 contributes to TGF-beta signaling through interaction with the type II receptor. J Cell Biol. 2007;178(2):201–208.

Kveiborg M, Frohlich C, Albrechtsen R, et al. A role for ADAM12 in breast tumor progression and stromal cell apoptosis. Cancer Res. 2005;65(11):4754–4761.

Kodama T, Ikeda E, Okada A, et al. ADAM12 is selectively overexpressed in human glioblastomas and is associated with glioblastoma cell proliferation and shedding of heparin-binding epidermal growth factor. Am J Pathol. 2004;165(5):1743–1753.

Rao VH, Kandel A, Lynch D, et al. A positive feedback loop between HER2 and ADAM12 in human head and neck cancer cells increases migration and invasion. Oncogene. 2012;31(23):2888–2898.

Carl-McGrath S, Lendeckel U, Ebert M, et al. The disintegrin-metalloproteinases ADAM9, ADAM12, and ADAM15 are upregulated in gastric cancer. Int J Oncol. 2005;26(1):17–24.

Mino N, Miyahara R, Nakayama E, et al. A disintegrin and metalloprotease 12 (ADAM12) is a prognostic factor in resected pathological stage I lung adenocarcinoma. J Surg Oncol. 2009;100(3):267–272.

Lin EY, Jones JG, Li P, et al. Progression to malignancy in the polyoma middle T oncoprotein mouse breast cancer model provides a reliable model for human diseases. Am J Pathol. 2003;163(5):2113–2126.

White DE, Kerpios NA, Zuo D, et al. Targeted disruption of beta1-integrin in a transgenic mouse model of human breast cancer reveals an essential role in mammary tumor induction. Cancer Cell. 2004;6(2):159–170.

Lafuste P, Sonnet C, Chazaud B, et al. ADAM12 and alpha9-beta1 integrin are instrumental in human myogenic cell differentiation. Mol Biol Cell. 2005;16(2):861–870.

Cao Y, Zhao Z, Gruszczynska-Biegala J, et al. Role of metalloprotease disintegrin ADAM12 in determination of quiescent reserve cells during myogenic differentiation in vitro. Mol Cell Biol. 2003;23(19):6725–6738.

Leyme A, Bourd-Boittin K, Bonnier D, et al. Identification of ILK as a new partner of the ADAM12 disintegrin and metalloprotease in cell adhesion and survival. Mol Biol Cell. 2012;23(17):3461–3472.