Metastatic role of galectin-1 in neuroendocrine cervical cancer

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Research Article

Keywords: metastasis, neuroendocrine cervical cancer, LGALS1

Posted Date: February 11th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1341512/v1

Abstract

Cervical cancer is ranked top ten in the ranking of female cancer and the metastasis of cervical cancer is one of the main causes of decreasing five-year-survival rates. Neuroendocrine cervical cancer belongs to rare type of cervical cancer and is one of the more malignant cancer type, compared to squamous cell carcinoma and adenocarcinoma. In the previous work, we have carried out proteomics-based 2D-DIGE and MALDI-TOF-MS to analyze the differentially modulated proteins in neuroendocrine cells (HM-1) and other types of cervical cancer cells (Caski, ME-180, HeLa). In which, galectin-1 (LGALS1) was selected as the candidate protein due to high expression of LGALS1 in HM-1 cells. In this study, use small interfering RNA (siRNA) to knockdown LGALS1 expression in HM-1and HM-2 which isolated from human tissue. The migration ability and proliferation ability of neuroendocrine cervical cancer cells are effectively inhibited. Also, the de-regulation of cell cycle progression was observed during LGALS1 silencing. As a result, LGALS1 affects the migration and proliferation ability in neuroendocrine cervical cancer. The protein is potential to be a candidate to detect and treatment of neuroendocrine cervical cancer.

1. Introduction

Neuroendocrine tumors initially deriving from nervous systems and endocrine occur frequently in the digestive system like intestine, as well as pancreas, and cervix [1]. Neuroendocrine cancers initially developing from nervous systems and endocrine take place frequently in the digestive system like intestine, as well as cervix and pancreas [1]. Neuroendocrine cervical cancer is a violent but infrequent form of cervical tumor with its incidence rate of below 3% of all cervical cancer patient cases. The greater part of neuroendocrine cervical cancer cases show advanced-stage diseases with lymph node metastasis as well as distinct metastasis demonstrating a high danger of disease recurrence as well as progression [2]. Based on a medica investigation, the ten-year survival rate of neuroendocrine cervical cancer patients with stage IB1 (tumor without metastasis of proximate lymph nodes) was 55%, in compare to 88% and 76% in squamous and adenocarcinoma patients, respectively [3]. Interestingly, even though there are several types of neuroendocrine cancer cases, these cases show common features in secreting specific hormones and granules as well as in cell morphology. Consequently, certain neuroendocrine tumor biomarkers including neuron-specific enolase, 5-hydroxyindoleacetic acid, chromogranin A, as well as synaptophysin have been reported. Nevertheless, the restricted quantity of these neuroendocrine cancer markers are not sufficient for clinical examinations [4].

LGALS1 is a β-galactoside binding protein that recognizes glyco-conjugates which located on cell surfaces as well as extracellular matrices [5]. LGALS1 is exceedingly expressed in nearly all types of malignant cancer cells and plays an essential pro-tumorigenic role within the tumor microenvironment [6]. The expression of LGALS1 has been well known in a number of different tumor types including bladder, thyroid, prostate, and ovarian carcinomas [7]. Interestingly, the expression of LGALS1 was shown to be positively correlated with the aggressiveness of tumors as well as the level of the metastatic phenotype; nevertheless, its exact effects on the disease outcome still remains indefinable. Therefore, it is essential

to investigate the role of the LGALS1 in cancer metastasis and progression, and examining the causal metastatic mechanisms in neuroendocrine cervical cancer.

In our previous study, we performed proteomics-based 2D-DIGE and MALDI-TOF-MS to investigate the differentially expressed proteins in neuroendocrine cells (HM-1) and other types of cervical cancer cells (Caski, ME-180, HeLa). In which, LGALS1 is one of the highly expressed proteins in HM-1 cells compared to the other cervical cancer cells [8]. In this study, we used small interfering RNA (siRNA) to knockdown LGALS1 expression in HM-1 and HM-2 cells which isolated from human tissues. The migration ability and proliferation ability of neuroendocrine cervical cancer cells are effectively inhibited. In addition, signal transduction of cytoskeleton regulation is inhibited. As a result, LGALS1 affects the migration and proliferation ability in neuroendocrine cervical cancer and might be a potential target in detection and treatment of neuroendocrine cervical cancer.

2. Materials And Methods

2.1. Chemicals and Reagents

Generic chemicals used in this study were analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). All primary and secondary antibodies were purchased from Genetex (Hsinchu, Taiwan) and GE Healthcare (Uppsala, Sweden), respectively.

2.2. Cell lines and cell cultures

HM-1 and HM-2 cells, both neuroendocrine cervical cancers obtained from McKay Hospital Hsinchu branch, are both used in the current study. HM-1 and HM-2 cells were originated from surgical removed stage two neuroendocrine cervical cancers of Taiwanese female cancer patients. Additionally, the other two cell lines: Hela cells (cervical adenocarcinoma) and ME-180 cells (cervical squamous carcinoma) were obtained from the American Type Culture Collection (Manassas, VA, USA). All of these cancer cell lines were grew in DMEM medium containing 10% fetal bovine serum, 2 mM of L-glutamine, 100 µg/mL of streptomycin, 100 IU/mL of penicillin (all purchased from Gibco-Invitrogen Corp., UK). All of these cancer cells were incubated in a humidified atmosphere containing 5% CO_2 at 37^{o}C.

2.3. MTT cell proliferation assay

The detailed MTT cell proliferation assay procedure has been reported in our previous study [9].

2.4. Transwell migration assay

Transwell assay was used to study the effect of cell migration in both HM-1 and HM-2 cells. The detailed assay procedure has been reported in our previous study [9].

2.5. Immunoblotting analysis

Immunoblotting analysis was performed to determine the protein expression levels and the detailed experimental method was described in our previous reports [10].

2.6. siRNA design, construction and transfection.

The siRNA against LGALS1 was synthesized by Invitrogen. The targeting sequence 5’- ACC TGA ATC TCA AAC CTG GAG AGT G-3’ (HSS106026, invitrogen) against LGALS1 was designed and cells were transfected with 20 nM of LGALS1 siRNA or the corresponding control (pGCsi-control) in serum free medium containing Lipofectamine RNAiMAX for 4 h, followed by recovery in medium containing 10% FBS for 24 h. The efficiency of siRNA knockdown was examined through immunoblotted against LGALS1.

3. Results

In our previous study, we reported a proteomics-based analysis of the differentially expressed proteins in HM-1 and other types of cervical cancer cells (Caski, ME-180, HeLa). LGALS1 was found to be over-expressed in HM-1 cells. In the current study, we examined the LGALS1 in HM-1, HM-2, Hela (a cervical adenocarcinoma line) and ME-180 (a cervical squamous carci-noma line). The results revealed that LGALS1 was over-expressed in HM-1 and HM-2 rather than in ME-180 and HeLa (Figure 1).

To investigate the metastatic and proliferative properties of LGALS1, a siRNA strain against LGALS1 constructed for LGALS1 knockdown experiments. The targeting sequence 5’- ACC TGA ATC TCA AAC CTG GAG AGT G-3’ was designed against LGALS1. Knockdown of LGALS1 with the 20nM of siLGALS1 showed more than 90% ability in reduction of LGALS1 protein expression, and this concentration was applied to further investigation (Figure 2A).

LGALS1 has been extensively described to be up-regulated in advanced metastatic cancers and the protein can promote cell proliferation but attenuate cell death [11]. However, little information was reported regarding the relationship between LGALS1 and neuroendocrine cervical cancer related invasion, and how LGALS1 functions in cell migration / invasion. To examine the biological role of LGALS1 in neuroendocrine cervical cancer related proliferation and migration, siRNA was used to attenuate the protein expression of LGALS1. In Figure 2B, we knockdown LGALS1 by siRNA for HM-1 cell proliferation analysis. HM-1 cells were transfected with 20 nM of LGALS1 siRNA for 4 h and recovered for at least 16 h in complete medium before analysis. Control MH-1 cells and siLGALS1 transfected HM-1 cells were both used to analyze the cell proliferation rate from day 1 to day 4. As results shown in Figure 2B, the qualified proliferation rates were considerably lower in siLGALS1-transfected MH-1 cells rather than in control MH-1 cells from days 2 to day 4.

We also investigated the biological effects of LGALS1 siRNA on cell cycle-associated regulators via immunoblotting assay. The protein expression levels of p27 and CDK6 were increased expressed in response to siLGALS1 treatment in both HM-1 and HM-2. In contrast, Both siRNA treated HM-1 and HM-2 showed down-regulated CDK2/4/6, cyclin D2 after siRNA treatment. Additionally, the phosphorylation level of retinoblastoma protein (Rb) was reduced in response to treatment with siLGALS1 in HM-1 (Figure 2C). Taken together, the deficiency of LGALS1 in HM-1 and HM-2 cells provoked up-regulation of the cell

cycle inhibitor p27 and silence of LGALS1 suppressed the neuroendocrine cervical cancer proliferation rate by up-regulating cell cycle inhibitory proteins.

We further investigated the effect of LGALS1 on modulating neuroendocrine cervical cancer migration by LGALS1 depletion. The results revealed knockdown of LGALS1 reduced the migration ability of MH-1 cells by 50% under chemotactic gradient when compared to untreated MH-1 cells (Figure 2D). To further examine whether LGALS1 knockdown interferes neuroendocrine cervical cancer migration and how LGALS1 acts, the cytoskeleton-regulatory protein, profilin, was examined and the result demonstrated that LGALS1 is able to attenuate neuroendocrine cervical cancer migration through inhibiting profilin-associated pathways (Figure 2E).

4. Discussion

A deeper insight into tumor metastasis mechanism is urgent to provide accurate diagnosis and therapy. Cervical cancer is ranked top in the ranking of female cancer and the metastasis of cervical cancer is one of the main causes of decreasing survival rates. Wherein, neuroendocrine cervical tumor is a violent but rare form of cervical tumor which show at a high danger of disease development as well as recurrence. Based on our previous proteomic study, LGALS1 was selected as a potential disease marker for neuroendocrine cervical cancer.

Previous reports revealed that overexpressed LGALS1 was observed in numerous malignant tumors and LGALS1 was correlated with cancer invasiveness and progression in gastric cancer [12], thyroid cancer [13], colorectal cancer [14]. and breast cancer [15]. However, the functional roles of LGALS1 in tumor progression and metastasis of neuroendocrine cervical cancer are rare to be elucidated. Our results demonstrated that the decreased LGALS1 expression significantly reduced cell proliferation and blocked cell cycle progression in both HM-1 and HM-2 cells. These findings implicated LGALS1 in tumor progression as well as to be a potential independent prognostic factor for neuroendocrine cervical cancer. A preceding study indicated that LGALS1 knockdown by gene silencing significantly inhibited cell proliferation in epithelial ovarian cancer *in vitro* [16]. Another study showed that A2780-1A9 cells treated with OTX008, the LGALS1 inhibitor, lead to the suppression of the AKT and ERK1/2-dependent survival pathways to induce anti-proliferative effects [17]. These results were consistent with the effects of LGALS1 on neuroendocrine cervical cancer cell growth.

The cyclin-dependent kinases CDK4 and CDK6 have been reported to form complex with cyclin D to promote cell proliferation. The predominant cellular target of cyclin D-CDK4/6 complex is the Rb protein, which inhibits cell-cycle progression from G1 to S phase until its inactivation by phosphorylation [18]. Phosphorylated Rb might release E2F transcription factor which can regulate RNA processing, inhibition of apoptosis and proceeding cells into S-phase [19]. Moreover, cyclin E-CDK2 complex plays an important role in the G1-S phase transition through phosphorylation of Rb [20]. In addition, cyclin E-CDK2 complex has been reported to interact p27 leading to ubiquitin-dependent degradation of p27 [21]. Our results demonstrated knockdown of LGALS1 deregulated cell cycle progression and inhibited CDK2/4/6 activity

through up-regulation of p27 expression and inactivation of Rb function implying silencing of LGALS1 leads to block of cell cycle in neuroendocrine cervical cancer. Our studies also demonstrated silence of LGALS1 reduced profilin expression followed by reducing migration ability of neuroendocrine cervical cancer cells.

LGALS1 is found both on plasma membrane and in extracellular spaces, the physical interaction between LGALS1 and its associated partners concern downstream signaling events. GM1 ganglioside, extracellular matrix components, H-Ras and integrins are all recognized to be the binding partners of LGALS [5]. Previous studies also revealed that integrin α11β1 [22], integrin α5β3 (Leblanc R, Lee SC, David M, et al. Interaction of platelet-derived autotaxin with tumor integrin alphaVbeta3 controls metastasis of breast cancer cells to bone. Blood 2014; 124: 3141-3150.) and integrin α2β1 [23] played important roles in promoting cancer metastasis. Additionally, LGALS1 interacted with integrin α6β4 to facilitate cancer metastasis in lung cancer [24]. Based on these studies, we suggest that over-expression of LGALS1 probably activates intracellular signaling to promote neuroendocrine cervical cancer metastasis via integrin signaling pathways. Further studies will be imperative to investigate which types of integrins are involved in the binding of LGALS1, contributing to neuroendocrine cervical cancer metastasis.

In summary, LGALS1 was over-expressed in neuroendocrine cervical cancer. Silencing of LGALS1 can promote neuroendocrine cervical cancer cell apoptosis, cell survival as well as attenuate migration ability of the cells through inhibition of CDK2, CDK4, CDK6, Cyclin D1 and p-Rb. Thus, LGALS1 is potentially to be a candidate to detect and treatment of neuroendocrine cervical cancer.

Abbreviations

Galectin-1: LGALS1

Declarations

Acknowledgments

This study was supported by Ditmanson Medical Foundation Chia-Yi Christian Hospital (R110-40). The author would like to thank Amber Kao for proofreading and editing of the manuscript.

All authors contributed to the study conception and design and all authors read and approved the final manuscript. The authors declare that they have no conflict of interest.

Research involving human participants and/or animals

Not applicable.

Informed consent

Not applicable.

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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Figures

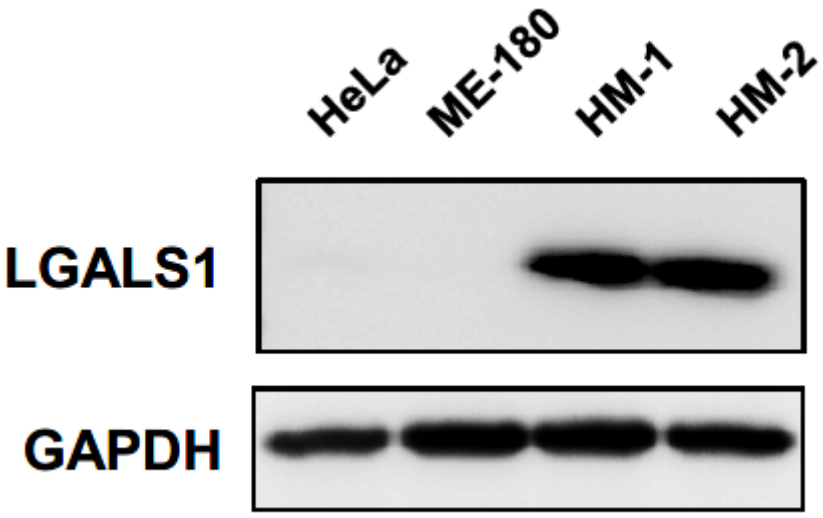

Figure 1

Properties of LGALS1 in representative cervical cancer cell lines. LGALS1 expression in Hela cells, ME-180 cells, HM-1 cells and HM-2 cells.

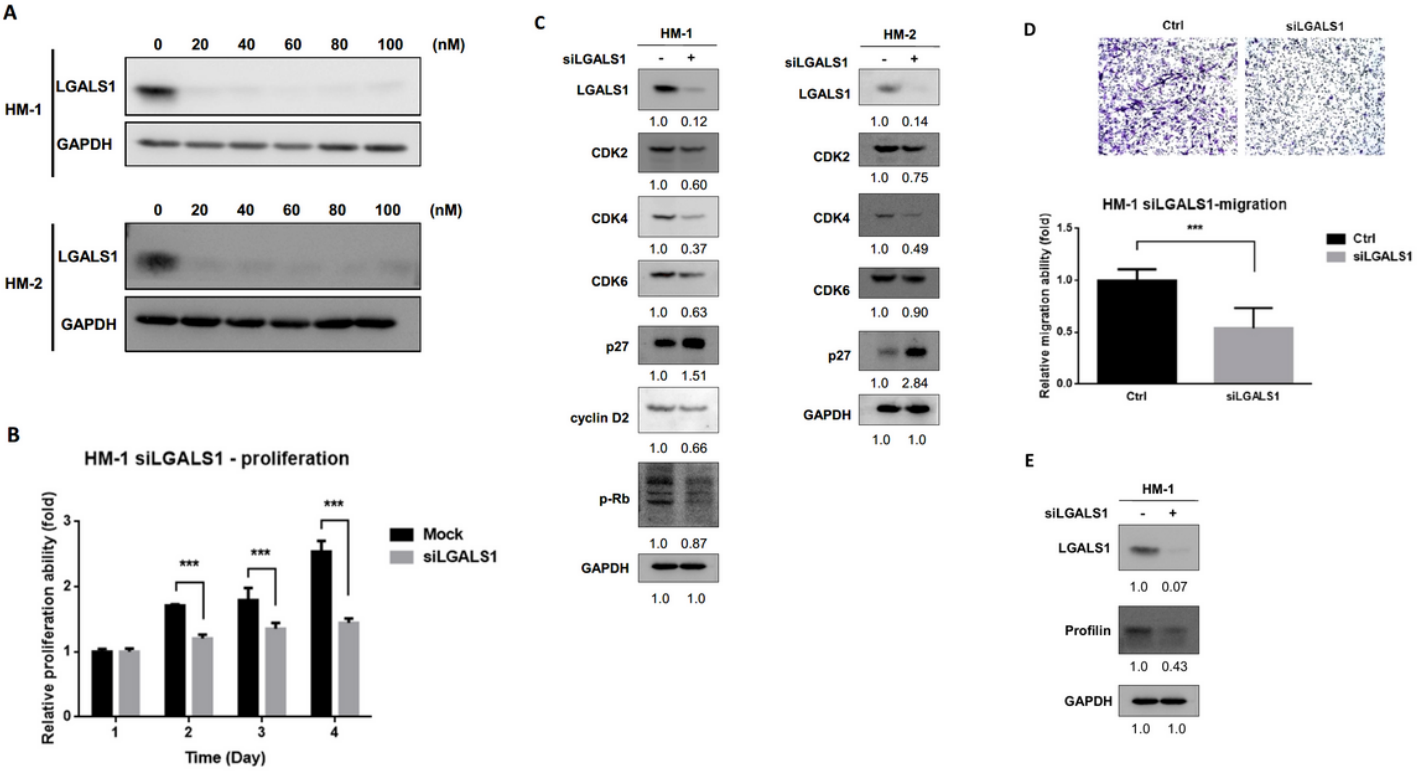

Figure 2

Effects of LGALS1 silence on cell proliferation and cell migration in HM-1 cells and HM-2 cells. (A) HM-1 cells and HM-2 cells were treated with differential amount of siLGALS1, and the knock-down effects of siLGALS1 were determined through immunoblotting. GAPDH level was used as a loading control. **(B)** Untreated or siLGALS1-treated HM-1 cells were seeded and incubated for 24 h and 48 h, followed by performing MTT assay. Error bars denote mean ± SEM (n = 3). ***p<0.001. **(C)** Representative images of the cell cycle-regulating proteins (CDK2, CDK4, CDK6, cyclin D2, p27 and pRb) normalized to GAPDH in siCtrl- or siLGALS1-transfected HM-1 cells and HM-2 cells. The protein expression values were quantified in relation to the siCtrl-transfected HM-1 cells and HM-2 cells. **(D)** Cells were transfected with siLGALS1 and recovered for 24 h. Cells were seeded into upper chamber without Matrigel in serum-free media. After 18 h, the migratory cells were fixed, stained by crystal violet and viewed microscopically. The crystal violet was dissolved in ethanol/acetic acid solution and quantified. Mock was regarded as negative control. Error bars denote mean ± SEM (n = 3). ***p<0.001. **(E)** HM-1 cells were transfected with siLGALS1 and recovered for 24 h. Immunoblotting analysis of profilin on cytoskeletal regulation pathway was performed. The GAPDH protein was served as protein loading controls for normalization.

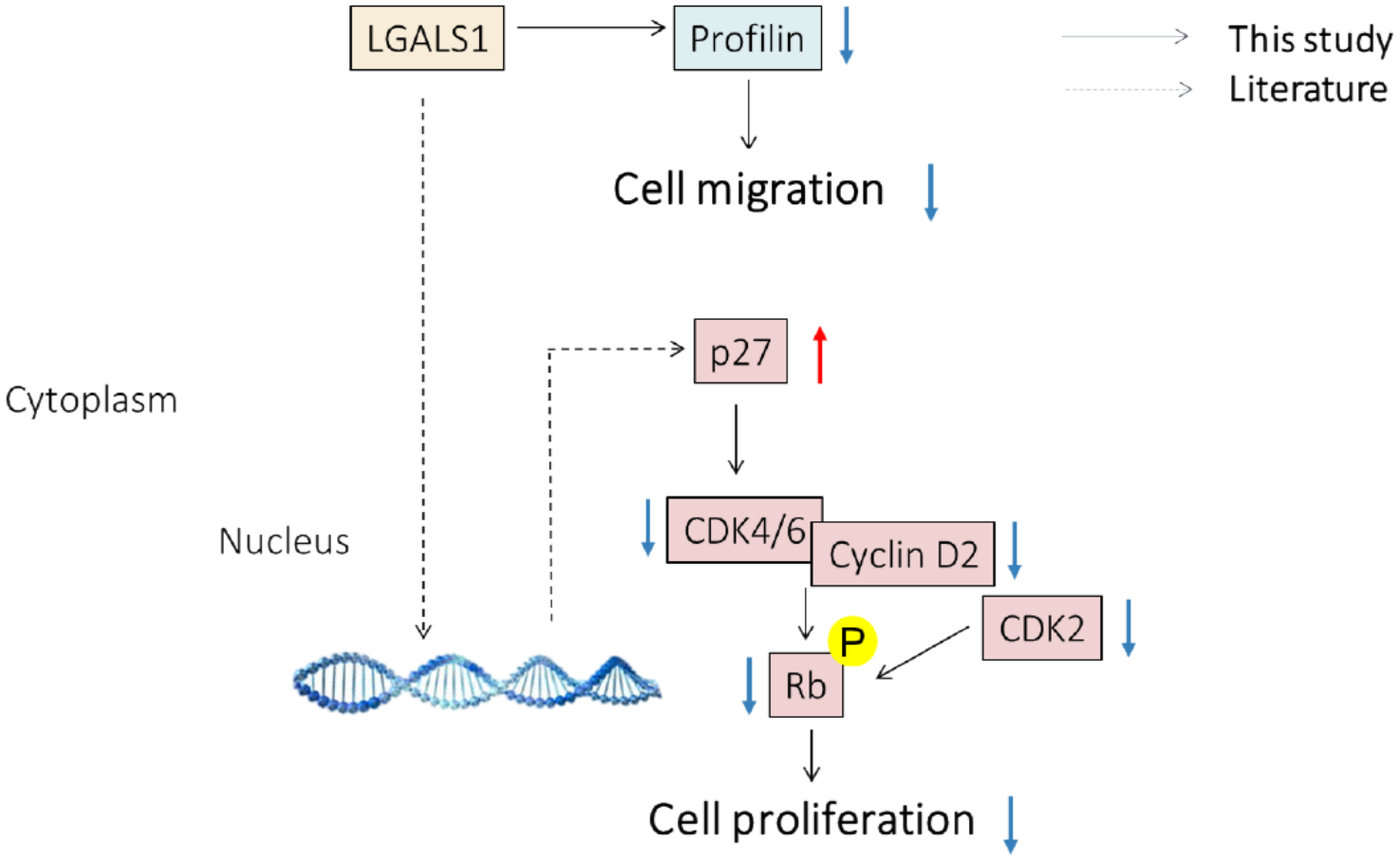

Figure 3

The molecular mechanism of LGALS1 on neuroendocrine cervical cancer. Silencing of LGALS1 might enhance p27 expression and reduced cell proliferation through down-regulation of CDKs and cyclin D2

followed by inactivated Rb tumor suppresor protein. In addition, LGALS1 knockdown reduced migration ability of neuroendocrine cervical cancer cells. The black solid lines means the direct evidences from this study. The black dashed lines means the evidences from published literatures.