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ORIGINAL ARTICLE

Axl glycosylation mediates tumor cell proliferation, invasion and lymphatic metastasis in murine hepatocellular carcinoma

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Abstract

AIM: To investigate the effects of Axl deglycosylation on tumor lymphatic metastases in mouse hepatocellular carcinoma cell lines.

METHODS: Western blotting was used to analyze the expression profile of Axl glycoprotein in mouse hepatocellular carcinoma cell line Hca-F treated with tunicamycin and PNGase F 3-(4,5)-dimethylthiazol(-zyl)-3,5-diphenyltetrazolium bromide (MTT) assay, extracellular matrix (ECM) invasion assay (*in vitro*) and tumor metastasis assay (*in vivo*) were utilized to evaluate the effect of Axl deglycosylation on the Hca-F cell proliferation, invasion and lymphatic metastasis.

RESULTS: Tunicamycin and PNGase F treatment markedly inhibited Axl glycoprotein synthesis and expression, proliferation, invasion, and lymphatic metastasis both *in vitro* and *in vivo*. In the MTT assay, proliferation was apparent in untreated Hca-F cells compared with treated Hca-F cells. In the ECM invasion assay (*in vitro*), treated cells passed through the ECMatrix gel in significantly smaller numbers than untreated cells (tunicamycin 5 μg/mL: 68 ± 8 *vs* 80 ± 9, $P = 0.0222$; 10 μg/mL: 50 ± 6 *vs* 80 ± 9, $P = 0.0003$; 20 μg/mL: 41 ± 4 *vs* 80 ± 9, $P = 0.0001$); (PNGase F 8 h: 66 ± 7 *vs* 82 ± 8, $P = 0.0098$; 16 h: 49 ± 4 *vs* 82 ± 8, $P = 0.0001$; 24 h: 34 ± 3 *vs* 82 ± 8, $P = 0.0001$). In the tumor metastasis assay (*in vivo*), average lymph node weights of the untreated Hca-F group compared with treated Hca-F groups (tunicamycin 5 μg/mL: 0.84 ± 0.21 g *vs* 0.72 ± 0.19 g, $P = 0.3237$; 10 μg/mL: 0.84 ± 0.21 g *vs* 0.54 ± 0.11 g, $P = 0.0113$; 20 μg/mL: 0.84 ± 0.21 g *vs* 0.42 ± 0.06 g, $P = 0.0008$); (PNGase F 8 h: 0.79 ± 0.15 g *vs* 0.63 ± 0.13 g, $P = 0.0766$; 16 h: 0.79 ± 0.15 g *vs* 0.49 ± 0.10 g, $P = 0.0022$; 24 h: 0.79 ± 0.15 g *vs* 0.39 ± 0.05 g, $P = 0.0001$). Also, average lymph node volumes of the untreated Hca-F group compared with treated Hca-F groups (tunicamycin 5 μg/mL: 815 ± 61 mm^3 *vs* 680 ± 59 mm^3, $P = 0.0613$; 10 μg/mL: 815 ± 61 mm^3 *vs* 580 ± 29 mm^3, $P = 0.0001$; 20 μg/mL: 815 ± 61 mm^3 *vs* 395 ± 12 mm^3, $P = 0.0001$); (PNGase F 8 h: 670 ± 56 mm^3 *vs* 581 ± 48 mm^3, $P = 0.0532$; 16 h: 670 ± 56 mm^3 *vs* 412 ± 22 mm^3, $P = 0.0001$; 24 h: 670 ± 56 mm^3 *vs* 323 ± 11 mm^3, $P = 0.0001$).

CONCLUSION: Alteration of Axl glycosylation can attenuate neoplastic lymphatic metastasis. Axl N-glycans may be a universal target for chemotherapy.

Key words: Axl; Glycosylation; Hepatocellular carcinoma; Lymphatic metastasis

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INTRODUCTION

The receptor tyrosine kinases (RTKs) constitute a large family of transmembrane proteins that relay signals from extracellular growth factors into the cell[1,2]. The Tyro-Axl-Mer (TAM) subfamily shares the vitamin K-dependent ligand Gas6 (growth arrest specific 6). TAM receptors contain a combination of two immunoglobin-like domains and dual fibronectin type Ⅲ repeats in the extracellular region, and a cytoplasmic kinase domain[3,4]. The TAM receptors regulate a diverse range of cellular responses including cell survival, proliferation, autophagy, migration, angiogenesis, platelet aggregation, and natural killer cell differentiation[4].

The Axl receptor (also called UFO, Tyro7, and Ark) is a RTK originally identified as a transforming gene in chronic myeloid leukemia[5,6]. Axl is expressed in various organs, including the brain, suggesting its involvement in mesenchymal and neural development[7,8]. Axl has been shown to have transforming potential when overexpressed during development. Axl overexpression is clearly associated with invasiveness and metastasis in several cancer cell types, including myeloid leukemia[6,9], esophageal[10], metastatic lung[11], metastatic colon[12], renal cell[13], prostate[14], breast[15], gastric[16], and thyroid[17] cancers. Axl also affects multiple pathways in angiogenesis[11]. Thus, Axl may play an important role in tumor progression, although its mechanism remains unknown.

Protein glycosylation is one of the major types of posttranslational modifications that has profound biological implications[18,19]. Specific changes in the glycosylation pattern of cell surface glycoproteins have been shown to correlate with metastatic efficiency in tumor cells[20]. In particular, protein N-glycosylation is one of the most prominent biochemical alterations in tumorigenesis and metastatic spread[21,22]. A cell surface transmembrane glycoprotein, little is known about the mechanism of Axl deglycosylation.

The mouse hepatocellular carcinoma cell line Hca-F is highly aggressive, with a metastasis rate over 80%. Hca-P, on the other hand, has a lymphatic metastasis rate of less than 30%. Both cell lines are derived from 615-mice ascites-type hepatocellular carcinoma cells. Hca-F and Hca-P cells metastasize only to lymph nodes, and not extrahepatic organs. However, the relationship between Axl glycosylation and lymphatic metastasis of mouse hepatocellular carcinoma cells remains unclear.

Our aim was to investigate whether Axl glycosylation regulates lymphatic metastasis. We demonstrated a possible correlation, based upon regulation of Axl glycosylation in mouse hepatocellular carcinoma cells.

MATERIALS AND METHODS

Cell culture and animals

Mouse hepatocellular carcinoma cell lines Hca-F and Hca-P, grown and stored in our institution (Department of Pathology, Dalian Medical University) were cultured in 90% Roswell Park Memorial Institute (RPMI)-1640 (Gibco) and supplemented with antibiotics (1 × penicillin/streptomycin 100 U/mL, Gibco) and 10% fetal bovine serum (FBS) (Gibco). Cells were incubated at 37 ℃ in a humidified atmosphere containing 5% CO_2. 615-mice (8 wk old males) were obtained from the Experimental Animal Center of Dalian Medical University.

Whole protein extract

10^7 cells were centrifuged at room temperature at 1000 × *g* for 10 min. Cells were rinsed twice with phosphate buffered saline (PBS) at 1000 × *g* for 5 min, and lysed with a protease inhibitor cocktail (whole protein extraction kit KGP2100, KeyGEN). Cells were suspended on a swing bed at 4 ℃ for 15 min, and centrifuged at 4 ℃ at 14 000 × *g* for 15 min. Protein concentration of the whole cells was measured with a bicinchoninic acid protein assay kit (KGPBCA, KeyGEN).

Western blotting analysis

Western blotting analysis was performed to evaluate Axl (with or without tunicamycin or PNGase F treatment) protein levels. Extracted proteins were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto polyvinylidene fluoride membranes (Pall Corporation). After blocking for 2 h with 5% skimmed milk in PBS containing 0.1% Tween 20 (PBST), membranes were incubated with rabbit anti-mouse Axl polyclonal antibody (Santa Cruz Biotech Inc., 1/200 diluted) overnight in 5% powdered skim milk buffer, washed thrice with PBS with 0.1% Tween 20, and then incubated with secondary antibody anti-rabbit-HRP (Santa Cruz Biotech Inc., 1/3000 diluted). Glyceraldehyde-3-phosphate dehydrogenase antibody (Santa Cruz Biotech Inc., 1/200 diluted) was used as controls. All blot analysis was performed with a ECL Western blotting kit (Amersham Biosciences, United Kingdom).

Tunicamycin treatment

To inhibit N-linked glycosylation of newly synthesized proteins, Hca-F cells were washed once with PBS and cultivated for 12 h in fresh culture media (90% RPMI 1640 supplemented with antibiotics) with or without tunicamycin (Sigma Aldrich, St. Louis, MO) in a dose-dependent manner (0 μg/mL, 5 μg/mL, 10 μg/mL, or 20 μg/mL). Cells were washed with PBS and subjected to Western blotting analysis, 3-(4,5)-dimethylthiazol(-zyl)-3,5-diphenyltetrazolium bromide (MTT), migration *in*

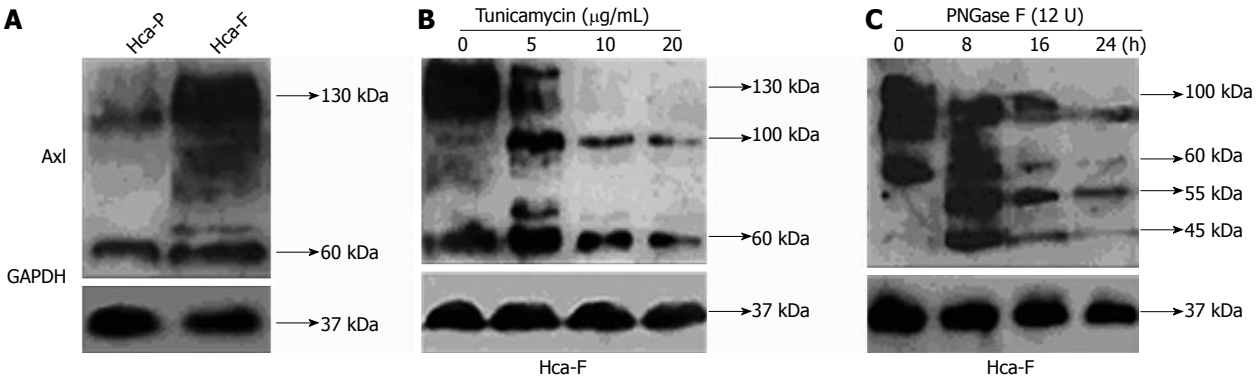

Figure 1 Expression profile of Axl glycoprotein in mouse hepatocellular carcinoma cell lines. A: Axl glycoprotein levels by Western blotting analysis in Hca-P and Hca-F cell lines. Relative signal intensities of Axl protein were compared with GAPDH by LabWorks (TM ver4.6, UVP; BioImaging Systems), ($^{a}P < 0.05$ *vs* untreated Hca-F cells); B: Hca-F cells were treated with 0 μg/mL, 5 μg/mL, 10 μg/mL, and 20 μg/mL tunicamycin for 12 h. Total protein extracts were loaded for each sample; C: Hca-F cell protein was deglycosylated with 12 units of PNGase F in lysis buffer. Probes were incubated at 37 ℃ in a time-dependent manner (0 h, 8 h, 16 h, 24 h). Protein was separated on a gel for Western blotting Analysis. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis, proteins were transferred to a polyvinylidene fluoride membrane, and were detected by rabbit anti-mouse Axl polyclonal antibody. GAPDH blotting was used as the control. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

vitro, and tumor metastasis *in vivo* assays.

PNGase F treatment

To remove N-glycans, protein fractions (100 μg) from Hca-F cells were deglycosylated with 25 units of PNGase F (Elizabethkingia meningoseptica; Sigma Aldrich, St. Louis, MO) in lysis buffer. Probes were incubated for 8 h, 16 h and 24 h at 37 ℃. The reaction was terminated with Laemmlie's sample buffer and proteins were separated on a gel as described earlier.

For deglycosylation of membrane proteins, intact Hca-F cells were incubated with 25 units of PNGase F for 24 h, washed, and treated as described for the MTT, migration *in vitro*, and tumor metastasis *in vivo* assays.

3-(4,5)-dimethylthiazol(-zyl)-3,5-diphenyltetrazolium bromide assay

10^6 cells in 200 μL RPMI 1640 were seeded in duplicate into 96-well culture plates, and 100 μL MTT (5 mg/mL, Sigma) was added at 24 h, 48 h, 72 h, 96 h, and 120 h, respectively. After 4 h incubation at 37 ℃ in 5% CO_2, 100 μL/well DMSO (final concentration 25%, Gibco) was pipetted to solubilize the formazan product for 30 min at room temperature. Absorbency (490 angstroms) was measured using a microplate reader (Bio-Rad).

In vitro extracellular matrix invasion assays

Cell invasion *in vitro* was demonstrated using 24-well transwell units (Corning, NY, United States) with a 8 μm pore size polycarbonate filter coated with ECMatrix gel (Chemicon) to form a continuous thin layer[23]. Cells (3×10^5) were harvested in serum-free medium containing 0.1% BSA and added to the upper chamber. The lower chamber contained 500 μL RPMI 1640. Cells were incubated for 24 h at 37 ℃, 5% CO_2 incubator. At the end of incubation, cells on the upper surface of the filter were completely removed with a cotton swab. The filters were fixed in methanol and stained with Wright-Giemsa. Cells invading the matrigel that reached the lower surface of the filter were counted with light microscopy at a magnification of 400 ×. Samples were acquired in triplicate and data expressed as the average cell number in 5 fields.

In vivo tumor metastasis assay

Forty eight 615-mice were provided with sterilized food and water and equally divided into eight groups. 10^7 Hca-F cells (with or without tunicamycin or PNGase F treatment) were subcutaneously inoculated into the footpads. After 3 wk, mice were sacrificed and their axillary lymph nodes were isolated, weighed, and photographed.

Statistical analysis

Each assay was performed at least three times. Data were presented as the mean ± SD. Statistical differences between test groups was assessed by one-way analysis of variance and Scheffe's test for post hoc analysis. A *P*-value of less than 0.05 was considered statistically significant. SPSS version 13.0 software was used for statistical analysis.

RESULTS

Expression profile of Axl glycoprotein in mouse hepatocellular carcinoma cell lines

Axl glycoprotein relative expression was determined by Western blotting analysis using whole-cell extracts (Figure 1A). Axl expression varied among cell lines, with higher and lower levels in Hca-F and Hca-P cells, respectively (Figure 1A, $P < 0.05$).

Tunicamycin, an inhibitor of endogenous N-linked glycosylation of newly synthesized proteins, was used to inhibit Axl glycosylation of Hca-F cells. Treatment in a dose dependent manner (0 mg/mL, 5 mg/mL, 10 mg/mL, and 20 mg/mL) for 12 h showed N-linked glycosylation to be highly sensitive to tunicamycin inhibition (Figure 1B).

Axl appears as broad bands, with molecular weights ranging from 60 kDa to 140 kDa. With tunicamycin treatment, 130 kDa Axl band density decreased, 60 kDa

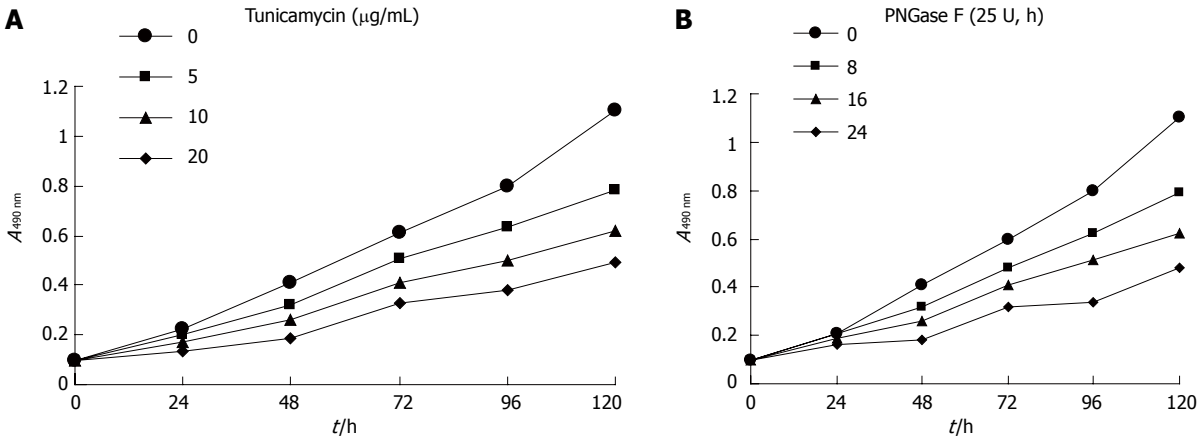

Figure 2 Axl deglycosylation effects on cell proliferation *in vitro*. Hca-F cells were exposed to tunicamycin or PNGase F and harvested at 24 h, 48 h, 72 h, 96 h, and 120 h. Cell proliferation was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Decreased proliferative ability was detected in cells treated with tunicamycin (A) or PNGase F (B), compared with untreated Hca-F cells. Data was obtained in triplicate.

band density increased, and a 100 kDa band appeared. As the dose of tunicamycin increased, the 130 kDa Axl band completely disappeared.

Whole protein aliquots extracted from Hca-F cells were exposed to exogenous PNGase F for deglycosylation (Figure 1C). 55 kDa and 45 kDa Axl bands appeared with PNGase F treatment. However, 60-140 kDa Axl band density significantly decreased. These results suggest that the N-glycosylation process for Hca-F cells responded to tunicamycin and PNGase F treatment.

Axl deglycosylation reduces cell proliferation in vitro

Hca-F cells treated with tunicamycin or PNGase F were measured for proliferative activity. Proliferation was apparent in untreated Hca-F cells compared with treated Hca-F cells (Figure 2A, B). Thus, Axl deglycosylation inhibited Hca-F cell proliferation *in vitro*.

Axl deglycosylation alters invasiveness of Hca-F cells in vitro

To examine whether Axl deglycosylation affects invasiveness of Hca-F cells, we performed *in vitro* ECMatrix gel analysis. We found that untreated and tunicamycin (Figure 3A) or PNGase F (Figure 3B) treated Hca-F cells or PNGase F had differing abilities to pass through an ECMatrix coated filter; therefore, the numbers of invading cells were unequal. Treated cells with tunicamycin passed through the ECMatrix gel in significantly smaller numbers than untreated cells (5 μg/mL: 68 ± 8 *vs* 80 ± 9, $P = 0.0222$; 10 μg/mL: 50 ± 6 *vs* 80 ± 9, $P = 0.0003$; 20 μg/mL: 41 ± 4 *vs* 80 ± 9, $P = 0.0001$). Similar results were shown with PNGase F treated Hca-F cells at 8 h, 16 h, and 24 h compared with untreated cells (66 ± 7 *vs* 82 ± 8, $P = 0.0098$; 49 ± 4 *vs* 82 ± 8, $P = 0.0001$; 34 ± 3 *vs* 82 ± 8, $P = 0.0001$). These results indicated that Axl deglycosylation reduced the invasiveness of Hca-F cells *in vitro*.

Axl deglycosylation inhibits the metastatic ability of Hca-F cells to peripheral lymph nodes in vivo

To further evaluate whether Axl deglycosylation was essential for tumor lymphatic metastasis *in vivo*, we tested the effect of Axl deglycosylation on the metastatic ability of Hca-F cells in mice peripheral lymph nodes. Treated and untreated Hca-F cells were injected in the footpads of 615-mice. After 3 wk' inoculation, a significant reduction in positive lymph nodes in the deglycosylation groups was observed, compared with untreated controls (Figure 4). Average lymph node weights of the untreated Hca-F group compared with dose-adjusted tunicamycin treated Hca-F groups (5 μg/mL: 0.84 ± 0.21 g *vs* 0.72 ± 0.19 g, $P = 0.3237$; 10 μg/mL: 0.84 ± 0.21 g *vs* 0.54 ± 0.11 g, $P = 0.0113$; 20 μg/mL: 0.84 ± 0.21 g *vs* 0.42 ± 0.06 g, $P = 0.0008$) (Figure 4A left). The average lymph node volumes of these groups were 815 ± 61 mm^3 *vs* 680 ± 59 mm^3, $P = 0.0613$; 815 ± 61 mm^3 *vs* 580 ± 29 mm^3, $P = 0.0001$; 815 ± 61 mm^3 *vs* 395 ± 12 mm^3, $P = 0.0001$ (Figure 4A right).

The average lymph node weights in the untreated Hca-F compared with PNGase F treated groups were: 8 h: 0.79 ± 0.15 g *vs* 0.63 ± 0.13 g, $P = 0.0766$; 16 h: 0.79 ± 0.15 g *vs* 0.49 ± 0.10 g, $P = 0.0022$; 24 h: 0.79 ± 0.15 g *vs* 0.39 ± 0.05 g, $P = 0.0001$ (Figure 4B left). The average lymph node volumes of these groups were: 670 ± 56 mm^3 *vs* 581 ± 48 mm^3, $P = 0.0532$; 670 ± 56 mm^3 *vs* 412 ± 22 mm^3, $P = 0.0001$; 670 ± 56 mm^3 *vs* 323 ± 11 mm^3, $P = 0.0001$ (Figure 4B right). These results demonstrate Axl deglycosylation may reduce Hca-F cells to peripheral lymph nodes *in vivo*.

DISCUSSION

Axl has garnered attention because of its high expression in many tumor cells, and its key role in neoplastic invasion and metastasis. In this study, we demonstrated Axl protein expression varied based on antineoplastic treatment of mouse hepatocellular carcinoma cell lines Hca-F and Hca-P. We found Axl protein expression to be higher in Hca-F cells, which have high lymphatic metastasis potential compared with Hca-P cells, which have low lymphatic metastasis potential. This confirms previ-

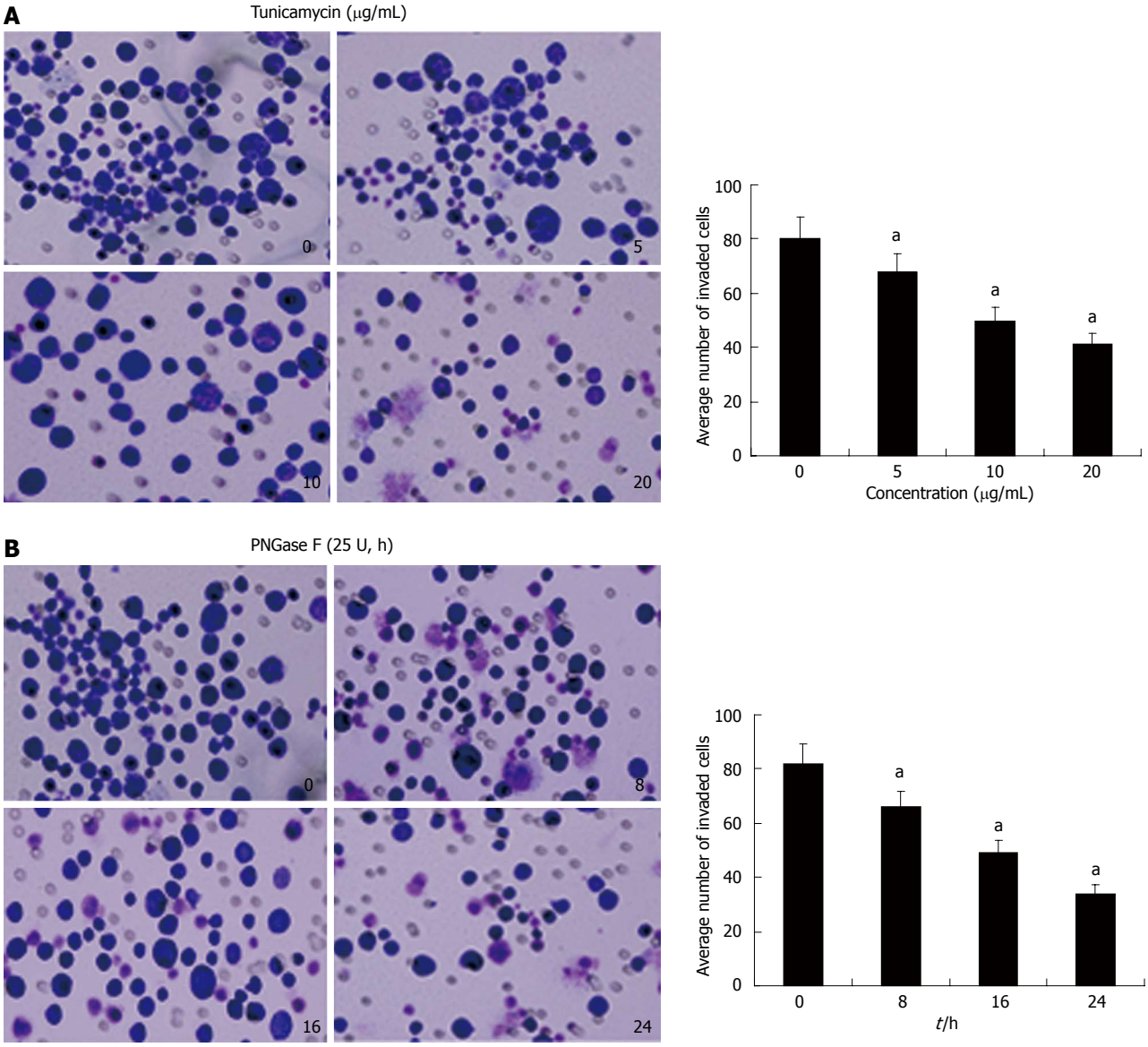

Figure 3 Axl deglycosylation alters the invasive ability of Hca-F cells *in vitro*. *In vitro* ECMatrix gel analysis. Wright-Giemsa staining results of the lower surface filter showed that the cells passed through the filter and attached to the lower side (400 ×). The average number of cells invading the filter was counted. Cells treated with tunicamycin (A) or PNGase F (B) were significantly less invasive ($^aP < 0.05$ *vs* untreated Hca-F cells) than untreated Hca-F cells. Data was obtained in triplicate.

ously reported findings of Axl overexpression in highly invasive lung adenocarcinoma cell lines, compared with their less invasive counterparts[11]. This suggests that high Axl expression may be associated with tumor lymphatic metastasis, and that Axl may be associated with tumor metastatic potential.

In our study, we achieved Hca-F deglycosylation with two methods. First, we inhibited N-glycan biosynthesis with tunicamycin; secondly, we extracted protein in the presence of PNGase F enzyme, which digests N-glycans. Both treatments resulted in significant effects on cell surface N-glycans by Western blotting assays.

Among post-translational modification reactions involving proteins, glycosylation is the most common; nearly 50% of all proteins are glycosylated[24]. Alterations of glycan structures are frequently observed in various cancer cells[25]; and this appears to be one association in cancer invasion and metastasis. We found Axl deglycosylation to be a possible factor in tumor progression, including cell proliferation, invasion, and lymphatic metastasis. In this study, we detected a significant inhibition of proliferation and invasion in Axl deglycosylated Hca-F cells *in vitro*, by both MTT and extracellular matrix assays. These results confirmed prior reports that cell proliferation requires growth factors signalling through cell surface glycoprotein receptors, which may be inactive when underglycosylated[26]. Although our findings support the role of Axl deglycosylation in reducing cell proliferation and invasion *in vitro*, its mechanism had not been elucidated. Further experiments showed that Axl deglycosylation led to a significant reduction in metastatic lymph node burden *in vivo*. These results were consistent previous reports of changes in N-linked oligosaccharide branching associated with malignancy and metastasis[27].

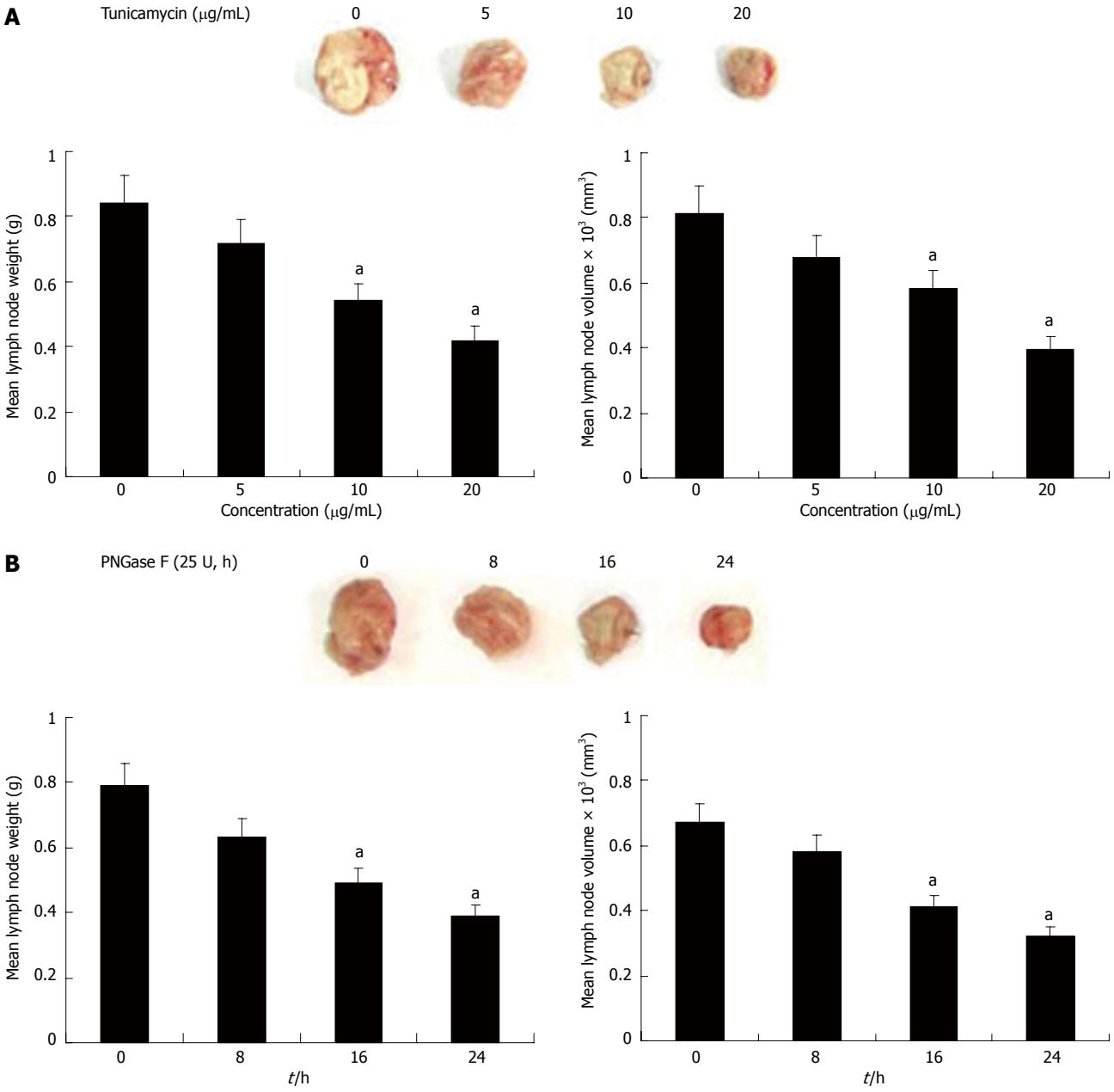

Figure 4 Axl deglycosylation inhibits the ability of Hca-F cells to metastasize to peripheral lymph nodes *in vivo*. Untreated and treated Hca-F cells were injected into the footpads of 615-mice. After 3 wk' inoculation, the mice were sacrificed and axillary lymph nodes isolated, weighed, measured, and photographed. A significant reduction in mean tumor weight ($n = 6$) of Axl tumor deglycosylation was observed, as compared with untreated Hca-F cells ($^aP < 0.05$ *vs* untreated Hca-F cells).

Some authors reported that the addition of exogenous Gas6 mediated the migration and invasion of Hca-F cells both *in vitro* and *in vivo* through the Axl pathway[28]. RNAi-mediated knockdown of Axl expression decreased the ability of YAP-expressing MIHA cells and of the primary HCC cell line to proliferate and invade[29]. In our study, we were unable to elucidate the mechanism by which Axl deglycosylation inhibits lymphatic metastasis in murine Hca-F cells. However, in many glycoproteins, N-linked oligosaccharides contribute to the folding, stability, and biological function of adhesion molecules and growth factor receptors on cell surfaces[30-32]. An increasing body of evidence indicates that glycoprotein glycans are involved in the regulation of cellular functions, including cell-cell communication and signal transduction[33,34]. The products of N-acetylglucosaminyltransferase (GnT)-IV, GnT-V and 1,6-fucosyltransferase (1,6-FucT) are all increased in hepatocellular carcinoma[35]. The presence of 1,6-GlcNAc structures in N-glycans and the expression of GnT-V, which catalyzes the addition of the 1,6-branching, were shown to promote metastasis[36-39]. At the very least, these reports demonstrate the relationship between metastasis and N-glycans to be extremely complicated. This area requires additional research.

In conclusion, we have found a role of Axl glycosylation in mediating tumor cell proliferation and invasion, and have provided the first evidence that Axl deglycosylation is required for lymphatic metastasis in murine

hepatocellular carcinoma cell lines. These results may at least partially explain the role of Axl glycosylation in the promotion of lymphatic metastasis. This study may provide new insights into regulatory mechanisms of mouse hepatocellular carcinoma with lymphatic metastasis.

COMMENTS

Background

Axl has been shown to have transforming capability when overexpressed. Prior studies have revealed Axl overexpression to be clearly associated with cancer invasiveness and metastasis. Axl also has multiple effects in angiogenesis. While Axl may play an important role in tumor progression, its mechanisms of action have not been understood.

Research frontiers

The authors investigated the potential effect of Axl deglycosylation the regulation of tumor lymphatic metastasis in mouse hepatocellular carcinoma cell lines. The authors evaluated the expression profile of Axl glycoprotein in the mouse hepatocellular carcinoma cell line Hca-F, which was treated with tunicamycin and PNGase F. Furthermore, the authors analyzed the effect of Axl glycosylation by tunicamycin and PNGase F treatment in Hca-F cells with regards to proliferation, invasion, and lymphatic metastasis both *in vitro* and *in vivo*.

Innovations and breakthroughs

Protein N-glycosylation is increasingly being recognized as one of the most prominent biochemical alterations in tumorigenesis and metastatic spread. However, as a cell surface transmembrane glycoprotein, little is known about Axl deglycosylation and its mechanism of action. Axl glycosylation was attenuated by tunicamycin and PNGase F to determine the effect on Hca-F cell proliferation, invasion, and lymphatic metastasis.

Applications

The authors have found the role of Axl glycosylation in mediating tumor cells proliferation, invasion and provided the first evidence that deglycosylation of Axl is required for metastasis of hepatocellular carcinoma cells to lymph nodes. This study may provide new insights into regulatory mechanisms of mouse hepatocellular carcinoma with lymphatic metastasis.

Terminology

The Axl receptor (also named UFO, Tyro7, and Ark) is a receptor tyrosine kinase (RTK) originally identified as a transforming gene in chronic myeloid leukemia. The RTKs constitute a large family of transmembrane proteins that relay signals from extracellular growth factors into the cell.

Peer review

In this study, the effect of Axl deglycosylation on lymphatic metastasis was investigated in mouse hepatocellular carcinoma cell lines. Differing Axl expression levels were found in mouse Hca-F and Hca-P cell lines, which are characterized by high and low metastatic potential, respectively. A decrease in Axl glycosylation by tunicamycin or PNGase F treatment resulted in a reduced proliferation, invasion, and lymphatic metastasis, both *in vitro* and *in vivo*. This work is potentially relevant in understanding hepatocellular carcinoma.

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