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Integrin expression and glycosylation patterns regulate cell-matrix adhesion and alter with breast cancer progression

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ABSTRACT

Integrins are the major cell adhesion glycoproteins involved in cell-extracellular matrix (ECM) interaction and metastasis. Further, glycosylation on integrin is necessary for its proper folding and functionality. Herein, differential expression of integrins viz., αvβ3 and αvβ6 was examined in MDA-MB-231, MDA-MB-468 and MCF-10A cells, which signify three different stages of breast cancer development from highly metastatic to non-tumorigenic stage. The expression of αvβ3 and αvβ6 integrins at mRNA and protein levels was observed in all three cell lines and the results displayed a distinct pattern of expression. Highly metastatic cells showed enhanced expression of αvβ3 than moderate metastatic and non-tumorigenic cells. The scenario was reversed in case of αvβ6 integrin, which was strongly expressed in moderate metastatic and non-tumorigenic cells. N-glycosylation of αvβ3 and αvβ6 integrins is required for the attachment of cells to ECM proteins like fibronectin. The cell adhesion properties were found to be different in these cancer cells with respect to the type of integrins expressed. The results testify that αvβ3 integrin in highly metastatic cells, αvβ6 integrin in both moderate metastatic and non-tumorigenic cells play an important role in cell adhesion. The investigation typify that N-glycosylation on integrins is also necessary for cell-ECM interaction. Further, glycosylation inhibition by Swainsonine is found to be more detrimental to invasive property of moderate metastatic cells. Conclusively, types of integrins expressed as well as their N-glycosylation pattern alter during the course of breast cancer progression.

1. Introduction

Integrins are major cell surface-adhesion glycoproteins composed of eighteen α and eight β subunits, which combine together to form 24 different types of integrins. These integrins can bind to different types of ECM ligand with partial overlapping [1,2]. Integrin αvβ3 is highly expressed in breast cancer cells as compared to normal breast epithelial cells [3]. Also, αvβ6 integrin is exclusively expressed in malignant epithelia, wound healing and developing tissue, and its expression is very less or rarely seen in the normal epithelia [4–7]. Integrin αvβ6 binds to fibronectin [8] and is highly expressed in colorectal carcinoma, non-small-cell lung carcinoma and MDA-MB-468 breast cancer cells [9–11]. Integrin β6 overexpression is reported to be correlated with EMT transition, invasion and metastasis [12,13]. Thus, dynamic modifications in the integrins could be conjoined with different progressive stages of cancer. N-glycosylation is an imperative post-translation modification of secreted and cell surface proteins [14]. Reports from the literature authenticate the fact that altered glycosylation on integrins, amends the cell adhesion properties of cancer cells [15]. It is hypothesized that modifications in integrin expression and their glycosylation pattern could play a crucial role in invasion, migration and metastasis by changing the adhesion of cells to ECM.

Swainsonine (SW) is a potent inhibitor of various α-mannosidases, especially α-mannosidase II, and thus inhibits protein glycosylation. Anti-cancerous activity of SW has been reported in many cancers elsewhere [16–21]. SW also reduces the survival of cancer cells and inhibits lung carcinoma A549 xenograft tumor [22]. Likewise, it promotes apoptosis in oesophageal squamous cell

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carcinoma cells *in vitro* and *in vivo* through activation of mitochondria [23]. However, effect of SW on highly metastatic breast cancer cells (MDA-MB-231), moderate metastatic cancer cells (MDA-MB-468) and non-tumorigenic breast cells (MCF-10A) has not been investigated so far.

Herein, we assessed cell adhesion and invasion property of αvβ3 and αvβ6 integrins as well as role of N-glycosylation on these integrins in breast cancer cells of two different stages of disease and non-transformed breast cells. Effect of SW alkaloid on the invasive properties of highly and moderate metastatic and non-tumorigenic cell lines was also investigated. The outcomes of the study recommend that all three cell lines have different cell adhesion properties and integrin expression pattern, which could have role in breast cancer progression.

2. Materials and methods

2.1. Materials

Function blocking integrin antibodies; integrin αvβ3 and αvβ6 were purchased from Millipore, USA. For western blotting integrin αv antibody from Millipore/Chemicon, and integrin β3 and integrin β6 antibodies from Abcam were used. HRP conjugated anti-mouse and anti-rabbit secondary antibodies and Swainsonine were obtained from Sigma, USA.

2.2. Cell lines and culture conditions

Human breast carcinoma MDA-MB 231 cells were grown in DMEM-F12 medium and MDA-MB 468 cells were grown in DMEM high glucose medium (Gibco, USA) containing 10% FBS and 1% antibiotic-antimycotic solution. MCF10A cells were grown in Mammary Epithelial Basal Medium (MEBM) supplemented with 0.4% bovine pituitary extract (BPE), 0.1% hEGF, insulin, hydrocortisone and 1% antibiotic-antimycotic solution. Cell lines were maintained in a humidified atmosphere at 37 °C with 5% CO2 in incubator.

2.3. Cell adhesion assay

Cell adhesion assay was performed with fibronectin coated 24-well plates pre-blocked with 1% BSA for 30 min. SW treated (10 and 20 μM) and untreated cells were allowed to adhere on plate. After 30 min of incubation at 37 °C, each well of plate was washed thrice with 1X PBS to remove unattached cells. The cells were fixed, washed and stained with crystal violet solution followed by washing and air-drying. Five-seven independent microscopic fields were counted for each group of experiment under 100× magnification with phase contrast microscope.

2.4. Function Blocking Assay

Briefly, cell suspension was incubated in the presence or absence of antibody against αvβ3 or αvβ6 or both of these integrins for 30 min. After incubation cells were allowed to adhere to pre-blocked (1% BSA for blocking) fibronectin-coated well plate for 30 min. Plates were further processed as described above.

2.5. Invasion assay

Cells were treated with 10 and 20 μM concentrations of SW for 24 h. Further cells were harvested and washed twice in serum-free media and counted using hemocytometer. To measure invasive property 40,000 cells in 500 μl serum-free media were seeded in upper chamber of invasion transwells and incubated for 17 h. Lower chambers were filled with 500 μl complete media containing 10% FBS. After incubation media was aspirated-out and inserts swabbed with cotton swab to remove non-invaded cells as well as matrigel on upper side. Invaded cells on the lower side were fixed and stained with 0.5% crystal violet solution. Cells were destained and inserts were air dried. Insert membranes were mounted on glass slides. Dried cells were counted at 400× magnification under phase contrast microscope (Olympus, Japan).

2.6. Semi-quantitative RT-PCR

Total RNA was isolated from cells using TRIzol reagent (Life Technologies) and cDNA was synthesized with the help of oligo (dT) primers and reverse transcriptase. Primers of integrin αv (F: 5′GTTTCAGTGTGCACCAGCAG3′ and R: 5′GCTCCCAGTTTGGAA TCGGA3′), β3 (F: 5′ACCAGTAACCTGCGGATTGG3′ and R: 5′CTCATTGAAGCGGGTCACCT3′), β6 (F: 5′TGTGGAAAAACCTGTAT CCCCT3′ and R: 5′AATCCACCTTCGGGTGTGTC3′) and GAPDH (F: 5′GCCTTCCGTGTCCCACTGC3′ and R: 5′CAATGCCAGCCCCAGCGTCA3′) were designed and used in PCR reaction. PCR amplification products were resolved by agarose gel electrophoresis.

2.7. Immunoblot analysis

SW treated (10 and 20 μM) and untreated cells were harvested after 24 h incubation and whole cell lysates were prepared [24]. Protein estimation was done by Bradford method. Samples were loaded on 10% denaturing SDS-PAGE gels and proteins were resolved. Proteins from the gel were transferred onto PVDF membrane and were blocked in blocking buffer for 1 h. Next, membrane was incubated with specific primary antibody followed by appropriate HRP-linked secondary antibody and processed for ECL detection.

2.8. Statistical analysis

Results were analyzed by Microsoft Excel and GraphPad Prism version 5 (La Jolla, CA) using unpaired Student t-test. Each experiment was done at least thrice with independent biological replicates. The results were represented as mean ± SE. P values < 0.05 were considered statistically significant.

3. Results

3.1. Role of αvβ3 and αvβ6 integrins in breast cancer cells adhesion to fibronectin

Adhesion of MDA-MB-231 cells on fibronectin mainly requires αvβ3 integrin as revealed by functional blocking assay (Fig. 1A and B), whereas MDA-MB-468 cells require αvβ6 integrin (Fig. 1C and D). Cell adhesion decreased in the presence of anti-αvβ3, anti-αvβ6 integrin and both antibodies by 69.7% ($P < 0.001$), 17.9% ($P < 0.01$) and 60.3% ($P < 0.001$) in MDA-MB-231 cells and by 7.3% ($P < 0.01$), 79.5% ($P < 0.001$) and 81.6% ($P < 0.001$) in MDA-MB-468 cells respectively (Fig. 1B and D). MDA-MB-231 cells showed high metastatic potential as compared to MDA-MB-468 cells as the binding was found greater in the former. Hence, enhanced metastatic potential of cells could be credited to high affinity of their integrins towards ECM.

3.2. Effect of swainsonine in cell adhesion

To study whether glycosylation is involved in cell adhesion, the cells were treated with 10 and 20 μM SW for 24 h. Cell adhesion was found decreased in the presence of SW at both doses (Fig. 2A–D).

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