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ORIGINAL ARTICLE

Studies on the role of goat heart galectin-1 as a tool for detecting post-malignant changes in glycosylation pattern

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Abstract Galectins are mammalian lectins established to play a crucial role in the progression of various cancer types by the virtue of their differential expression in normal and cancerous cells. In the present study, goat heart galectin-1 (GHG-1) was purified and investigated for its potential role in the detection of post-malignant changes in glycosylation pattern. When exposed to superoxide radicals generated from a pyrogallol auto-oxidation system, GHG-1 treated erythrocyte suspension released higher amount of oxyhemoglobin than the unagglutinated erythrocytes. The extent of erythrocyte hemolysis was found to be directly proportional to concentrations of hypochlorous acid. GHG-1 was used to detect the change in the β-galactoside expression pattern in erythrocyte membrane from human donors suffering from prostate and breast cancer. No significant change was observed in the hemolysis of lectin agglutinated erythrocytes collected from pre-operated breast cancer patients, whereas significant increase was observed in normal healthy control and post-operated samples. Findings of this study proclaim GHG-1 as an important tool for the detection of post-malignant changes in glycosylation pattern.

Abbreviations: Gal-1, galectin-1; GHG-1, goat heart galectin-1; HOCl, hypochlorous acid; OxyHb, oxyhemoglobin

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1. Introduction

Galectins are β-galactoside binding lectins established as potential target for cancer therapy (Hasan et al., 2007). Galectin-1 (Gal-1) has been reported to bind preferentially to ganglioside GM_1 on neuroblastoma cells and facilitate growth control (Kopitz et al., 2003; Robert et al., 2012) and also provide hydrophobic tails as interaction site for oncogenic

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H-Ras (Rotblat et al., 2004). The underlying mechanism to carry out this role is its glycan binding property present on cell membranes, thereby causing lysis of cells. A number of glycoconjugates expressed on the erythrocyte membranes have been reported to be altered in primary cancerous and metastatic conditions (Pugalendhi et al., 2010). Some noticeable alterations in the serum glycoconjugates have been observed in patients with various cancer types (Shetty et al., 2013). These alterations in glycoconjugates can act as excellent indicators for diagnostics, staging, prognostics, therapeutics, and detection of early recurrence in cancer (Baxi et al., 1991; Hernández-Hernández et al., 2006; Shetty et al., 2013). Owing to their multivalent sugar binding property, lectins have been used as an excellent tool for the detection of aberrant glycosylation related to various carcinomas and may provide useful diagnostic or prognostic information, thus contributing directly to cancer biology (Hernández-Hernández et al., 2006). The remarkable role played by galectins ranging from cell signaling to apoptosis make them potent tumorigenic molecules, and have been reported to be over-expressed quite often in cancerous cells and cancer associated stromal cells (Lahm et al., 2004). This altered expression of galectins correlates with the acquisition of metastatic phenotype and tumor aggressiveness, indicating toward the potential ability of galectins in the modulation of tumor progression thus influencing the outcome of the disease (Greco et al., 2004).

In the present study, goat heart galectin-1 (GHG-1) was purified (Ashraf et al., 2011) and investigated for the effect of pyrogallol and hypochlorous acid (HOCl) on the hemolysis of GHG-1 agglutinated erythrocytes. In our earlier study, we also reported that glycosylation plays a crucial role in maintaining the structural and functional integrity of GHG-1 (Ashraf et al., 2010a). Since erythrocytes of various carcinoma cells have been reported to show distinct glycosylation patterns which become a diagnostic index to examine the presence and proliferation of well known cancers, we also investigated the varied expression pattern of β-galactoside sugar residues on erythrocyte membrane of breast and prostate cancer patients using GHG-1 as a diagnostic tool.

2. Materials and methods

2.1. Reagents

Sephadex G100 and G50, molecular weight markers (14.4–97.4 kDa), coomassie brilliant blue (CBB) G-250 and R-250, sugars, pyrogallol and HOCl were purchased from Sigma Aldrich (St Louis, MO, USA). All other chemicals used were of analytical grade and were purchased from Qualigens Fine Chemicals and Merck India Ltd., India.

2.2. Isolation and purification of GHG-1

GHG-1 was isolated and purified essentially according to the methods used in our earlier studies on heart galectins (Ashraf et al., 2010a,b, 2011). The standard method of Lowry was used to estimate the protein concentration (Lowry et al., 1951). The method of two fold serial dilutions was used to determine the protein activity (Raz and Lotan, 1981).

2.3. Effect of GHG-1 on pyrogallol induced free radical damage to erythrocyte membrane

GHG-1 agglutinated erythrocyte suspensions (300 µl) were exposed to superoxide radicals generated from a pyrogallol auto-oxidation system (by adding 10 µl of 0.02 M pyrogallol solution freshly prepared in hydrogen peroxide) and incubated at 37 °C for 20 min. Erythrocytes were recovered by centrifugation at 3000 rpm for 5 min. Cells were then washed thrice and centrifuged with PBS 'B' and all the washings were pooled for analyzing the oxyhemoglobin (OxyHb) released. The released OxyHb concentration in the supernatant was measured by the equation of Winterbourn (1985).

$$[\text{OxyHb}] = (119\text{A}_{577}) - (39\text{A}_{630}) - (89A_{560})$$

2.4. Effect of HOCl on GHG-1 induced hemolysis of trypsinized rabbit erythrocytes

GHG-1 agglutinated erythrocyte suspensions (300 µl) were treated with varying concentrations of HOCl (50–350 µM) in PBS 'B' at 22 °C for 20 min. Cells were then washed thrice with excess of cold PBS 'B' and suspended in PBS 'B' as 10% suspension. The susceptibility of erythrocytes to HOCl induced oxidative damage was measured in terms of percent hemolysis as discussed above.

2.5. Differential hemolytic action of GHG-1 toward erythrocytes of breast and prostate cancer patients

Pre-operated and post-operated (7 days after surgery) heparinized venous blood samples from breast and prostate cancer patients (age > 45 years) were procured from the surgery outpatient department and male surgical wards of the Department of Surgery, Jawaharlal Nehru Medical College, Aligarh, India. Plasma was separated by centrifuging the blood sample at 5000 rpm for 5 min, the pellet containing erythrocytes was washed thrice with cold PBS 'B' and 4% RBC suspension was prepared. A trypsin solution (100 mg%) was then added to erythrocytes (0.1 ml of trypsin solution per ml of erythrocyte suspension) and incubated for 1 h at 37 °C. The trypsinized erythrocytes were washed four to five times with PBS 'B' and finally 8% erythrocyte suspension was prepared and percent hemolysis was determined as discussed above.

3. Results and discussion

Oxidative stress has been reported to enhance endothelial binding of lectins and activate the lectin complement pathway (Collard et al., 2001; Laderach et al., 2013), thus proclaiming an interesting correlation between lectins and oxidative stress. We thus investigated the effect of GHG-1 on pyrogallol induced free radical damage to erythrocyte membrane. Trypsinized rabbit erythrocyte suspension (8%) when treated with GHG-1 for 1 and 4 h and then exposed to superoxide radicals generated from a pyrogallol auto-oxidation system, released 28 and 36 µM oxyhemoglobin, respectively, in comparison with 6 µM oxyhemoglobin released by unagglutinated erythrocytes (Fig. 1). However, no release of oxyhemoglobin was observed in erythrocytes, which were neither treated with