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Research Article

Adhatoda vasica Leaves Protect Cell Surface Glycoconjugates Abnormalities During DMBA Induced Hamster Buccal Pouch Carcinogenesis.

*Manoharan S, Prabhakar MM

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar 608 002, Tamilnadu, India.

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ABSTRACT

Glycoproteins play an integral role in several biological functions including cell-cell interaction. Altered glycoproteins in the cell membrane or surface leads to malignant transformation. The main objective of the present study is to explore the protective effect of *Adhatoda vasica* on cell surface abnormalities during DMBA induced hamster buccal pouch carcinogenesis. Hamsters treated with DMBA alone for 14 weeks (thrice a week) in the buccal pouches resulted in well developed and well differentiated squamous cell carcinoma. Increased glycoprotein content was noticed in both tumor tissues and circulation of hamsters treated with DMBA alone. Oral administration of *Adhatoda vasica* ethanolic leaf extract (*AVELet*) at a dose of 100 mg/kg bw to hamsters treated with DMBA prevented the oral tumor formation as well as protected the DMBA- induced cell surface abnormalities. The present study thus concludes that *Adhatoda vasica* has the ability to protect the cell surface abnormalities induced by the carcinogen, DMBA, during oral carcinogenesis.

Key words: *Adhatoda vasica*, glycoconjugates, DMBA, oral cancer.

INTRODUCTION

Oral cancer causes significant morbidity and mortality worldwide and imposes burdens on the survival as well as life quality of the patients. Though this form of cancers affects 2-3 % of western populations, the incidence is very high in developing countries such as India, where it accounts for 40-50% of all cancers[1]. Tobacco and betel quid chewing along with alcohol abuse are pointed out as major risk factors of oral carcinogenesis[2]. Early diagnosis of oral cancer could only help to prevent or improve the life quality of the patients, despite recent advancements in chemotherapy and radiotherapy[3]. Hence, investigation of novel agents from medicinal plants with low toxicity or less side effects are still warranted not only to treat oral cancer at an early stage but also to improve the survival outcome of the patients.

Tumor cells exhibit several biochemical abnormalities as compared to their normal cellular counterpart[4]. One of the most significant abnormalities noticed in tumor cell surface is in the status of glycoproteins. Glycoproteins, proteins conjugated with oligosaccharide chain, play a pivotal role in several cellular mechanisms including cell adhesion and cell- cell communication[5]. Normal cells are converted into malignant ones if glycoproteins are abnormally expressed on the cell surface. Sialic acid and fucose, the two important glycoconjugates, play crucial role in neoplastic transformation[6]. Profound scientific studies clearly demonstrated abnormal levels of glycoproteins, especially sialic acid and fucose, in both human and experimental carcinogenesis[7-8].

Medicinal plants serve as an important resource for the treatment of several disorders including cancer. So far, around thirty anticancer drugs have been isolated from medicinal plants and are currently used in cancer treatment. Though several medicinal plants were tested for their anticancer efficacy, the anticancer potential of several other plants remains to be elucidated. *Adhatoda vasica*, is one such plant possesses a spectrum of bioactive principles to treat various diseases. The Indian traditional system of medicine like Ayurveda strongly recommends *Adhatoda vasica* to treat various disorders including cancer[9]. Experimental studies documented its hepatoprotective, anti-inflammatory, antimicrobial and anticancer properties[10-13]. Phytochemical analysis revealed the presence of quinazoline, vasicine, vasicinone, deoxyvasicine and phenolic compounds[14]. The major aim of the present study is to assess the protective effect of *Adhatoda vasica* on the status of cell surface glycoconjugates in DMBA induced oral carcinogenesis.

MATERIALS AND METHODS

Preparation Of Plant Extract: The *Adhatoda vasica* leaves were collected in and around Chidambaram, Cuddalore district, Tamilnadu, India, in August to September at the end of flowering season. The taxonomic identification of the plant was compared with the existing herbarium in the Botany department of Annamalai University. Five hundred

Author for correspondence

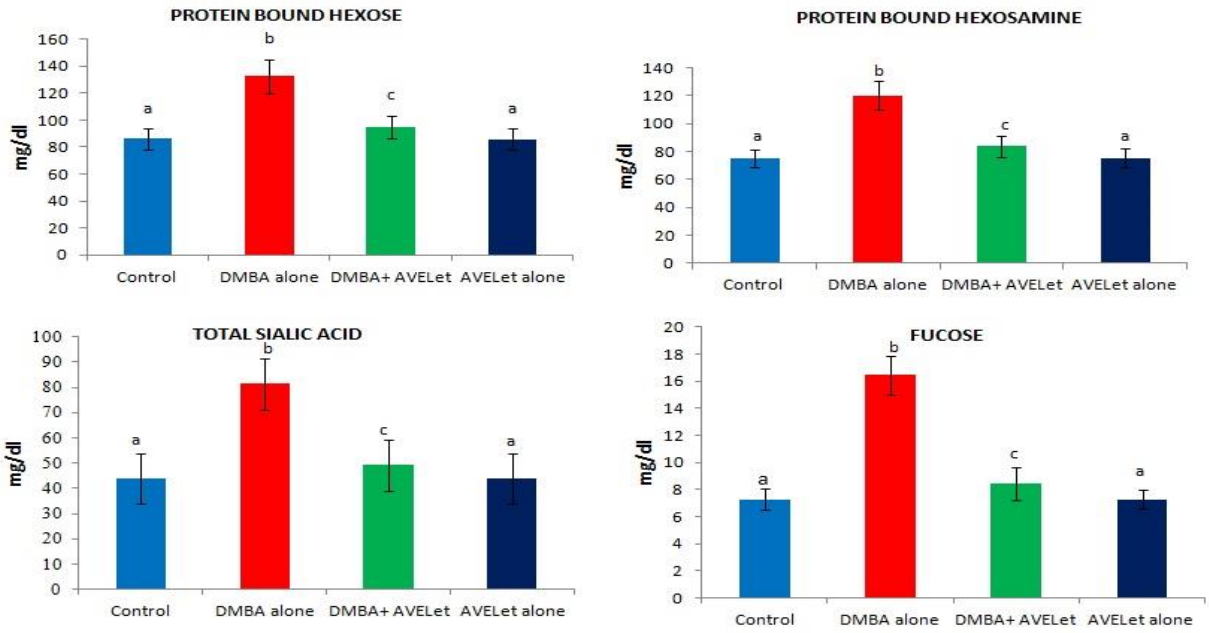

Fig. 1: The levels of glycoconjugates (protein-bound hexose, hexosamine, total sialic acid and fucose) in the plasma of control and experimental hamsters in each group.
Values are expressed as mean ± Standard deviation (S.D) for ten hamsters in each group. Values that do not share a common superscript between groups differ significantly at p < 0.05 (DMRT).

grams of dried, finely powdered *Adhatoda vasica* leaves were soaked with 1500 ml of 95% ethanol overnight. The residue obtained after filtration was again resuspended in equal volume of 95% ethanol for 48h and filtered again. The above two filtrates were mixed and the solvents were evaporated in a rotavapour at 40-50% under reduced pressure. A semisolid material (7%) obtained was stored at -4ºC for further experimental use. A known volume of the residual extract is suspended in distilled water and orally administered to the animals (100 mg / kg bw) by gastric intubation using force feeding tube during the experimental period. The above dose was selected on the basis of dose dependent studies.

Experimental Design: Forty male golden Syrian hamsters, 8 weeks old, weighing 80-120g, were obtained from National Institute of Nutrition, Hyderabad, India and maintained in Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. The institutional animal ethics committee [Reg.no:160/1999/CPCSEA], Annamalai University, Annamalainagar, approved the experimental design. The hamsters were divided into four groups of 10 hamsters in each. Group I hamsters served as the vehicle-treated control and painted with liquid paraffin alone three times a week for 14 weeks on their left buccal pouches. Groups II and III hamsters were painted with 0.5% DMBA in liquid paraffin three times a week for 14 weeks on their left buccal pouches. Group II hamsters received no further treatment. Group III hamsters received oral administration of *Adhatoda vasica* ethanolic leaf extract (*AVELet*) at a dose of 100 mg / kg bw, starting one week before the exposure to the carcinogen and was continued on days alternative to DMBA painting for 14 weeks (pre-initiation phase). Group IV hamsters received oral administration of *AVELet* (100 mg/ kg bw) alone throughout the experimental period. The experiment was terminated at the end of the 16th week (pre-initiation phase) and all hamsters were sacrificed by cervical dislocation. Biochemical studies were conducted in the plasma and buccal mucosa of control and experimental hamsters in each group.

Analysis of Glycoconjugates: The precipitate obtained after treating the plasma with 95% ethanol was used for the estimation of the protein-bound hexose[15], hexosamine[16], total sialic acid[17], and fucose[18]. The defatted tissues were treated with 0.1N H_2SO_4 and then hydrolyzed at 80°C for 1 hour. It was cooled and the aliquot was used for the sialic acid estimation. To the remaining solution, 0.1N sodium hydroxide was added and it kept in an ice bath for 1 hour and used for the estimation of protein-bound hexose and fucose.

Statistical Analysis: The data are expressed as mean ±SD. Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT). The results were considered statistically significant at $P \leq 0.05$.

RESULTS

Figures 1 and 2 depict the levels of glycoconjugates (protein-bound hexose, hexosamine, total sialic acid and fucose) in the plasma and buccal mucosa (protein-bound hexose, total sialic acid and fucose) of control and experimental hamsters in each group respectively. The levels of glycoconjugates in the plasma and buccal mucosa were significantly increased in hamsters treated with DMBA as compared to control hamsters. Oral administration of *Adhatoda vasica* ethanolic leaf extract (*AVELet*) at a dose of 100 mg/kg bw to DMBA-treated

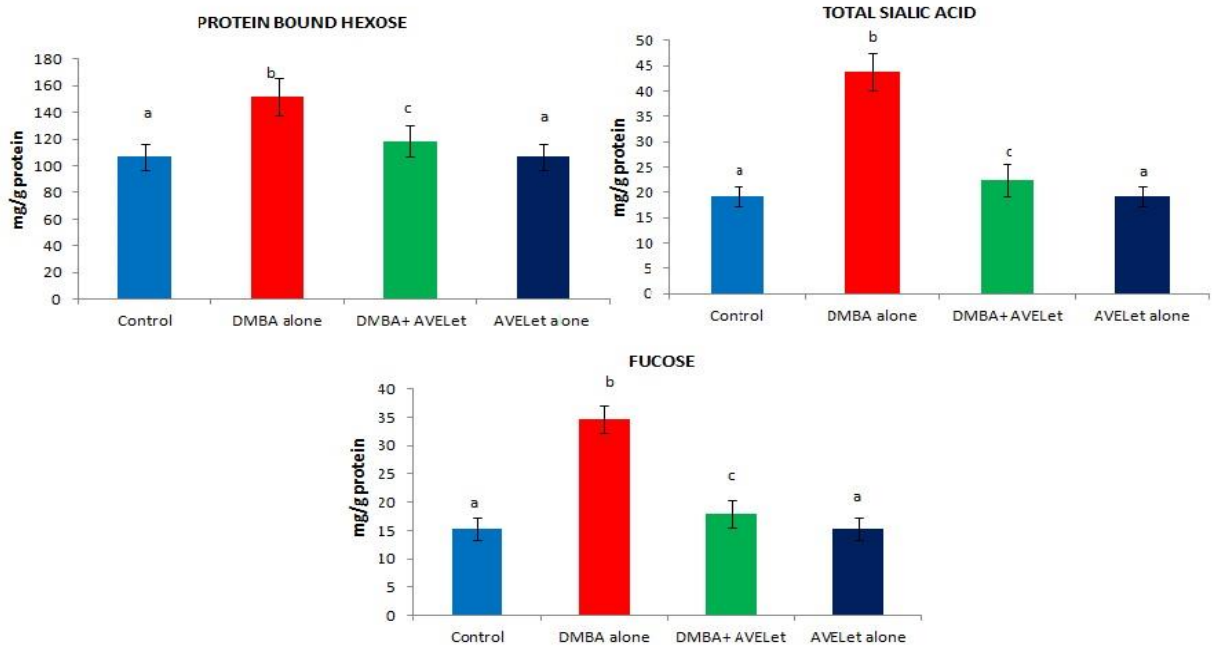

Fig. 2: The levels of glycoconjugates (protein-bound hexose, total sialic acid and fucose) in the buccal mucosa of control and experimental hamsters in each group.
Values are expressed as mean ± Standard deviation (S.D) for ten hamsters in each group. Values that do not share a common superscript between groups differ significantly at $p < 0.05$ (DMRT).

hamsters brought back the levels of the above-mentioned glycoconjugates to near normal levels. Hamsters treated with *AVELet* alone showed no significant difference in the levels of plasma and buccal mucosa glycoconjugates as compared to control hamsters.

DISCUSSION

A large number of cancer researchers focused the significance of glycoproteins as tumor specific markers[19-21]. Glycoconjugates have a vital role in many biological processes such as cell-cell recognition, cell growth and differentiation, and cell-matrix interaction[22]. Abnormal expression of glycoconjugates facilitates invasion and metastasis[23]. It has been reported that tumor cells secrete or express new glycoproteins moiety on their cell surface as well as glycoproteins are secreted into circulation from the destructed tumor tissues[24]. Atypical glycosylation has been recognized as a characteristic feature of oral malignant transformation[25]. Glycoconjugates status could help to assess the prognosis as well as monitoring the treatment strategy[26]. Cell surface glycoconjugates are abnormally increased if the cells are exposed to potent carcinogens.

DMBA induced cell surface abnormalities in relation to the expression of glycoconjugates are well documented[27]. Abnormal levels of glycoproteins in plasma and tumor tissues were reported in several cancerous conditions including oral cancer[28-30]. The levels of glycoconjugates are increased in a stepwise manner from stage I to stage IV of oral malignancies[31]. Tumor cells abnormally express sialic acid, a nine carbon sugar commonly known as N-acetyl neuraminic acid[32]. Extensive studies pointed out that tumor cells have two fold increase in sialic acid concentrations as compared to their normal cellular counterpart[33,34]. Fucose, the terminal moiety of glycoconjugates, is a five carbon sugar and plays a crucial role in malignant transformation. Increased concentration of fucose has been reported in plasma and tumor tissues of both human and experimental oral carcinogenesis[35,36]. A large number of cancer researchers pointed out that increased turnover of sialic acid and fucose in tumor tissues with subsequent leakage into circulation could account for increased levels of plasma fucose and sialic acid[37,38]. Increased levels of glycoconjugates in tumor tissues could be due to neosynthesis of glycoproteins in cancerous conditions. Increased levels of plasma glycoproteins are probably due to secretion from the destructed tumor tissues[39]. Increased levels of glycoconjugates may also be due to abnormal glycosylation, fucosylation and sialylation in the tumor tissues[40].

In the present study, we analysed the status of glycoproteins in the tumor tissues as well as in the circulation in the oral tumors bearing animals due to the fact that glycoproteins play significant role in cellular proliferation as well as in tumor invasion and metastasis. In the present study, oral administration of *Adhatoda vasica* ethanolic leaf extract at a dose of 100 mg/kg bw significantly decreased the levels of glycoconjugates in the buccal mucosa and plasma of tumor bearing hamsters. The present results thus indicate that *Adhatoda vasica* leaves has the potential to inhibit the activities of enzymes that are involved in the process of glycosylation, fucosylation and sialylation. The protective potential of *Adhatoda vasica* further implies that it significantly prevented the destruction of tissue damage by the carcinogen, DMBA.

The present study thus demonstrates the protective efficacy of *Adhatoda vasica* on cell surface

glycoconjugates abnormalities during DMBA induced oral carcinogenesis. Further studies are warranted to investigate the effect of *Adhatoda vasica* on the activities of enzymes involved in glycosylation, fucosylation and sialylation process during DMBA induced oral carcinogenesis.

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REFERENCES

1. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncology 2009; 45(4-5):309-316
2. Petti S, Masood M, Scully C. The magnitude of tobacco smoking-betel quid chewing-alcohol drinking interaction effect on oral cancer in South-East Asia. A meta-analysis of observational studies. PLoS One 2013; 8(11):e78999
3. More Y, D'Cruz AK. Oral cancer: review of current management strategies. The National Medical Journal of India 2013; 26(3):152-158
4. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144(5):646-674
5. Ohtsubo K, Marth JD. Glycosylation in cellular mechanisms of health and disease. Cell 2006; 126(5):855-867
6. Bose KS, Gokhale PV, Dwivedi S, Singh M. Quantitative evaluation and correlation of serum glycoconjugates: Protein bound hexoses, sialic acid and fucose in leukoplakia, oral sub mucous fibrosis and oral cancer. Journal of Natural Science, Biology and Medicine 2013; 4(1):122-135
7. Peracaula R, Barrabés S, Sarrats A, Rudd PM, de Llorens R. Altered glycosylation in tumours focused to cancer diagnosis. Disease Markers 2008; 25(4-5):207-218
8. Brooks SA, Carter TM, Royle L, Harvey DJ, Fry SA, Kinch C, Dwek RA, Rudd PM. Altered glycosylation of proteins in cancer: what is the potential for new anti-tumour strategies. Anti-Cancer Agents in Medicinal Chemistry 2008; 8(1):2-21
9. Singh RP, Padmavathi B, Rao AR. Modulatory influence of Adhatoda vesica (Justicia adhatoda) leaf extract on the enzymes of xenobiotic metabolism, antioxidant status and lipid peroxidation in mice. Molecular and Cellular Biochemistry 2000; 213(1-2):99-109
10. Claeson UP, Malmfors T, Wikman G, Bruhn JG. Adhatoda vasica: a critical review of ethnopharmacological and toxicological data. Journal of Ethnopharmacology 2000; 72(1-2):1-20
11. Bhattacharyya D, Pandit S, Jana U, Sen S, Sur TK. Hepatoprotective activity of Adhatoda vasica aqueous leaf extract on D-galactosamine-induced liver damage in rats. Fitoterapia 2005; 76(2):223-235
12. Chakraborty A, Brantner AH. Study of alkaloids from Adhatoda vasica Nees on their antiinflammatory activity. Phytotherapy Research 2001; 15(6):532-544
13. Singh B, Sharma RA. Anti-inflammatory and antimicrobial properties of pyrroloquinazoline alkaloids from Adhatoda vasica Nees. Phytomedicine 2013; 20(5):441-445
14. Khursheed A, Pathak D, Ansari SH. Phytochemical and Pharmacological Investigations on Adhatoda zeylanica (Medic.). A Review. Pharmacognosy Journal 2010; 2 (12):513-519.
15. Niebes P. Determination of enzymes and degradation product of glycosaminoglycan metabolism in the serum of health and various subjects. Clinica Chimica Acta 1972; 42:399-408.
16. Wagner WD. A more sensitive assay discriminating galactosamine and glucosamine in mixture. Analytical Biochemistry 1979; 94:369-394.
17. Warren L. Thiobarbituric acid and assay of sialic acid. Journal of Biological Chemeistry 1959; 30:171-180
18. Dische L, Shettles LB: Specific color reactions of methyl pentoses and spectrophotometric micromethod for their determination. Journal of Biological Chemeistry 1948; 175:595-604
19. Narimatsu H, Sawaki H, Kuno A, Kaji H, Ito H, Ikehara Y. A strategy for discovery of cancer glyco-biomarkers in serum using newly developed technologies for glycoproteomics. FEBS Journal 2010; 277(1):95-105
20. Kuzmanov U, Musrap N, Kosanam H, Smith CR, Batruch I, Dimitromanolakis A, Diamandis EP. Glycoproteomic identification of potential glycoprotein biomarkers in ovarian cancer proximal fluids. Clinical Chemistry and Laboratory Medicine 2013; 51(7):1467-1476
21. Reis CA, Osorio H, Silva L, Gomes C, David L. Alterations in glycosylation as biomarkers for cancer detection. Journal of Clinical Pathology 2010; 63(4):322-329
22. Hakomori S. Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)lipid metabolism. Cancer Research 1996; 56(23):5309-5318
23. Li M, Song L, Qin X. Glycan changes: cancer metastasis and anti-cancer vaccines. Journal of Biosciences 2010; 35(4):665-673
24. Yen TY, Macher BA, McDonald CA, Alleyne-Chin C, Timpe LC. Glycoprotein profiles of human breast cells demonstrate a clear clustering of normal/benign versus malignant cell lines and basal versus luminal cell lines. Journal of Proteome Research 2012; 11(2):656-667
25. Arnold JN, Saldova R, Galligan MC, Murphy TB, Mimura-Kimura Y, Telford JE, Godwin AK, Rudd PM. Novel glycan biomarkers for the detection of lung cancer. Journal of Proteome Research 2011; 10(4):1755-1764
26. Matsuda A, Kuno A, Matsuzaki H, Kawamoto T, Shikanai T, Nakanuma Y, Yamamoto M, Ohkohchi N, Ikehara Y, Shoda J, Hirabayashi J, Narimatsu H. Glycoproteomics-based cancer marker discovery

adopting dual enrichment with Wisteria floribunda agglutinin for high specific glycodiagnosis of cholangiocarcinoma. Journal of Proteomics 2013; 85:1-11
27. Manoharan S, Kavitha K, Balakrishnan S, Rajalingam K. Clerodendron inerme protects cellular integrity during 7,12-dimethylbenz[A]-anthracene induced hamster buccal pouch carcinogenesis. African Journal of Traditional, Complementary, and Alternative Medicine 2008; 5(2):213-222
28. Thakkar V, Patel P, Prajapati N, Kaur R, Nandave M. Serum Levels of Glycoproteins are Elevated in Patients with Ovarian Cancer. Indian Journal of Clinical Biochemistry 2014; 29(3):345-350
29. Bradley WP, Blasco AP, Weiss JF, Alexander JC Jr, Silverman NA, Chretien PB. Correlations among serum protein-bound carbohydrates, serum glycoproteins, lymphocyte reactivity, and tumors burden in cancer patients. Cancer 1977; 40(5):2264-2272
30. Ghosh M, Nayak BR. Serum sialic acid, fucose, sialic acid/fucose ratio as tumor markers in oral cancer. Annals of Dentistry. 1991; 50(2):33-45
31. Manoharan S, Padmanabhan M, Kolanjiappan K, Ramachandran CR, Suresh K. Analysis of glycoconjugates in patients with oral squamous cell carcinoma. Clinica Chimica Acta 2004; 339(1-2):91-96
32. Joshi S, Hegde AM, Rai K, Shetty S. Evaluation of salivary sialic acid levels in acute lymphoblastic leukemic children and its correlation with dental caries experience. Journal of Clinical Pediatric Dentistry 2013; 37(3):309-313
33. Uslu C, Taysi S, Akcay F, Sutbeyaz MY, Bakan N. Serum free and bound sialic acid and alpha-1-acid glycoprotein in patients with laryngeal cancer. Annals of Clinical and Laboratory Science 2003; 33(2):156-159
34. Warren L, Fuhrer JP, Tuszynski GP, Buck CA. Cell-surface glycoproteins in normal and transformed cells. Biochemical Society Symposium 1974; (40):147-157
35. Sawke NG, Sawke GK. Serum fucose level in malignant diseases. Indian Journal of Cancer 2010; 47(4):452-457
36. Parwani RN, Parwani SR. Quantitative evaluation of serum fucose in oral squamous cell carcinoma patients. Journal of Cancer Research and Therapeutics 2011; 7(2):143-147
37. Moriwaki K, Miyoshi E. Fucosylation and gastrointestinal cancer. World Journal of Hepatology 2010; 2(4):151-161
38. Kossowska B, Ferens-Sieczkowska M, Gancarz R, Passowicz-Muszyńska E, Jankowska R. Fucosylation of serum glycoproteins in lung cancer patients. Clinical Chemistry and Laboratory Medicine 2005; 43(4):361-369
39. Rao VR, Krishnamoorthy L, Kumaraswamy SV, Ramaswamy G. Circulating levels in serum of total sialic acid, lipid-associated sialic acid, and fucose in precancerous lesion and cancer of the oral cavity. Cancer Detection and Prevention 1998; 22(3):237-240.
40. Hauselmann I, Borsig L. Altered tumor-cell glycosylation promotes metastasis. Frontiers in Oncology 2014; 4:28