Lectin Histochemistry Shows WGA, PHA-L and HPA Binding Increases During Progression of Human Colorectal Cancer

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Abstract. *Background/Aim: Most colorectal carcinomas develop from an adenoma–carcinoma sequence to metastatic disease. The aim of the present study was to use lectins, carbohydrate-binding proteins, to detect changes in glycosylation during this malignant progression. Materials and Methods: Sections from normal colorectal mucosa, high-grade intraepithelial neoplasia, submucosal colorectal carcinoma and metastases from patients who underwent colorectal surgery were stained by lectins with different sugar specificities namely agglutins from Wheatgerm (WGA), Helix pomatia (HPA), Phaseolus vulgaris (PHA-L), Ulex europaeus (UEA-I), Sambucus nigra (SNA-I), Canavalia ensiformis (Con A), Galanthus nivalis (GNA) and Dolichos biflorus (DBA). Results: Binding patterns of all lectins except SNA-I, Con A and DBA changed during the adenoma–carcinoma sequence. Conclusion: The results indicate that lectins specific for mannose, N-acetylgalactosamine, N-acetylglucosamine, sialic acid, β-1,6-branched oligosaccharides and α-1-fucose may be associated with malignant progression.*

Colorectal cancer represents the second most common cancer-related cause of death in Germany (1). Most colorectal cancer cases are classified as adenocarcinomas (1), which almost exclusively develop from adenomas. This process is called the adenoma–carcinoma sequence and describes the transformation from healthy colorectal epithelial cells to adenoma and ultimately to carcinoma by genetic mutation (2). The process of metastasis formation is the most dangerous aspect of this malignant transformation process. It is a multi-step process which is still poorly understood. Circulating in the blood or in the lymphatic system, the tumor cells have to survive and, in the case of the hematogenous metastases, they have to attach to the endothelium at the site of the target organ (3-5). After attachment to the endothelium, the malignant cells have to cross the endothelial barrier and lodge in the connective tissues of the host organ to form a new tumor.

Studies have shown that lectins, carbohydrate-binding proteins of non-immunological origin (6, 7), can be used as prognostic markers in colorectal cancer (8), in gastric (9) and breast cancer (10), as well as in malignant melanoma (11). In particular, binding to the lectin Roman snail *Helix pomatia* agglutinin (HPA) is indicative of poor prognosis in colon cancer (8). Primary colon cancer cells which bound HPA had a greater tendency to become metastatic than did primary colon cancer cells which did not bind to HPA (8). This association between HPA binding and metastasis formation has been also validated in breast and colon cancer xenograft models, where HPA-positive cells proved to be metastatic, while HPA-negative ones were not (12). Both studies show that the carbohydrate residues of cancer cells are very important in the process of metastasis. However, we still do not exactly know why lectin-binding tumors preferably metastasize and others do not (8) – HPA binds specifically to *N*-acetlygalactosamine, which some authors hypothesize serves as a ligand for selectins (13). This might explain how cancer cells establish in a new organ.

In addition to HPA, the lectin from *Phaseolus vulgaris* leukoagglutinin (PHA-L) which binds to β1-6 branched oligosaccharides was also found to have a functional importance for the metastatic process in colon (14), breast (14) and prostate (15) cancer.

The main aim of this study was to investigate the binding pattern of eight lectins with different nominal carbohydrate-binding specificities and to make a comparison between these eight different lectins. In distinction from previous

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Key Words: Adenoma–carcinoma sequence, colon cancer, glycosylation, lectins, malignant progression, metastasis.

Table I. *Lectins, their species of origin, nominal sugar-binding specificity and their commercial source.*

Lectin	Species of origin	Sugar-binding specificity	Supplier catalog code
Con A	*Canavalia ensiformis*	Mannose	Sigma, C2272
DBA	*Dolichos biflorus*	α-*N*-Acetylgalactosamine	Sigma, L6533
GNA	*Galanthus nivalis*	Mannose	Vector, B-1245
HPA	*Helix pomatia*	*N*-Acetylgalactosamine/-glucosamine	Sigma, L6512
PHA-L	*Phaseolus vulgaris*	β-1-6–Branched oligosaccharides	Vector, B-1115
SNA I	*Sambucus nigra*	α 2,6-Linked *N*-acetylneuraminic acid	Vector, B-1305
UEA I	*Ulex europaeus*	α-1-Fucose	Sigma, L8262
WGA	*Triticum vulgaris*	*N*-Acetylglucosamine/sialic acid	Vector, B-1025

studies (14, 16-22), we explored the binding of these eight different lectins in a series of biopsies reflecting the entire spectrum of malignant progression, ranging from normal colorectal tissue to metastatic carcinoma. In particular, we wanted to investigate if there is a change in the lectin binding pattern during particular stages of malignant progression.

Materials and Methods

Histochemistry. Paraffin sections of lesions from patients who underwent colorectal surgery were analyzed. There were 46 sections of carcinomas, 22 sections of metastases, 14 sections of adenomas and 14 sections of normal colon examined in this study. The extraction of human tissue in surgical operations and its additional use for purpose of the research is a standard practice in Germany and therefore needed no approval by the Ethical Committee of the university.

The sections were stained using eight different biotinylated lectins (Table I) using the avidin–biotin alkaline phosphatase complex for visualization of the binding sites (23). Sections were deparaffinized, brought to distilled water through a series of graded ethanolic solutions and were rinsed with lectin buffer (for 10 l: 60.57 g Trizma Base Sigma-Aldrich, 87.09 g NaCl, 200 ml of 2 N HCl, 2.032 g $MgCl_2$, 1.01 g $CaCl_2$) and were incubated with 0.1% trypsin in lectin buffer at 37˚C for 10 min. Subsequently, the sections were rinsed under running tap water for 5 min and were then twice incubated in lectin buffer for 5 min each. This procedure was followed by the incubation of the sections with 100 to 200 μl of a 10 μg/ml biotinylated lectin solution for one hour at room temperature (for lectins see Table I). Negative controls and the inhibition of lectin staining with hapten sugars were included. The sections were afterwards rinsed with TRIS-buffered saline (TBS; for 10 l: 60.57 g Trizma Base Sigma-Aldrich, 87.09 g NaCl, 200 ml of 2N HCl) and incubated three times for 5 min in TBS followed by an incubation with the ABC complex (Vectastain Laboratories, Peterborough, United Kingdom) for 30 min. The sections were then washed again with TBS three times for 5 min each. After the final washing with TBS, the sections were incubated in the development solution for 30 min in the dark. For the development solution, 300 mg sodium nitrite was dissolved in 7.5 ml distilled water; 300 μl of Neufuchsin-HCl (Sigma-Aldrich, St.Louis, Missouri, United States of America) solution and 150 ml of TBS/HCl were added until a pH of 8.24 was reached. Naphthol-AS (Sigma-Aldrich, St. Louis, Missouri, USA) was dissolved in 750 μl dimethylformamide and this was added to the above solution. Finally, 200 μl Tween 20 (Sigma-Aldrich) and levamisol (Sigma-Aldrich) were added. After remaining 30 min in the development solution, reaction was stopped by rinsing the sections under running tap water for 5 minutes. Then the sections were washed with distilled water for an additional 5 min, and were counterstained with Mayers hemalum (Merck Millipore, Billerica, Massachusetts, United States of America) for 5 sec. Then the sections were blued under running tap water for 5 min and briefly rinsed with distilled water for 10 sec. The sections were then dehydrated and mounted with Eukitt (Kindler, Freiburg).

Scoring of lectin histochemistry results. The lectin binding was scored as follows: strong: 80-100% of the cells were intensely labeled, moderate: 21-79% moderately labeled and weak: <5-20% weakly labeled. We recorded whether the apical membrane or other external membranes were stained, whether the goblet cells were stained and whether there was any lectin binding to connective tissue. These aspects were noted separately.

Results

In normal colon and in high-grade intraepithelial neoplasia, WGA mostly bound to the epithelium only weakly or moderately (Figure 1). The brush border in normal colon and high-grade intraepithelial neoplasia was labeled in nearly all cases, while the basal membrane was decorated in 60% of the cases in normal colon and in 64% of the cases in carcinoma *in situ*. The goblet cells in normal colon and in high-grade intraepithelial neoplasia did not stain, and the connective tissue was labeled moderately. In carcinomas, 47% of the sections bound WGA strongly. In metastases, WGA mostly (50% of the sections) bound strongly. In carcinomas and metastases, the brush border was labeled in nearly all cases; the remaining basolateral membrane never stained. The connective tissue was stained moderately.

PHA-L gave similar results: as shown in Figure 2, the majority of healthy colon and high-grade intraepithelial neoplasia exhibited little PHA-L binding (20% and less). The brush border of nearly all cases was PHA-L labeled, while other membranes did not stain. In some cases, the goblet cells were stained, but the connective tissue showed no PHA-L binding. Cells of carcinomas and metastases principally bound this lectin more frequently with moderate or even

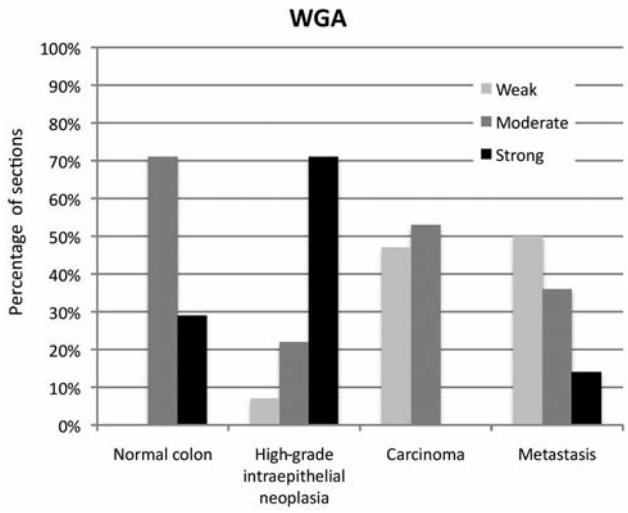

Figure 1. *Binding of wheatgerm agglutinin (WGA) to normal colon, high-grade intraepithelial neoplasia, carcinoma and metastasis by staining frequency.*

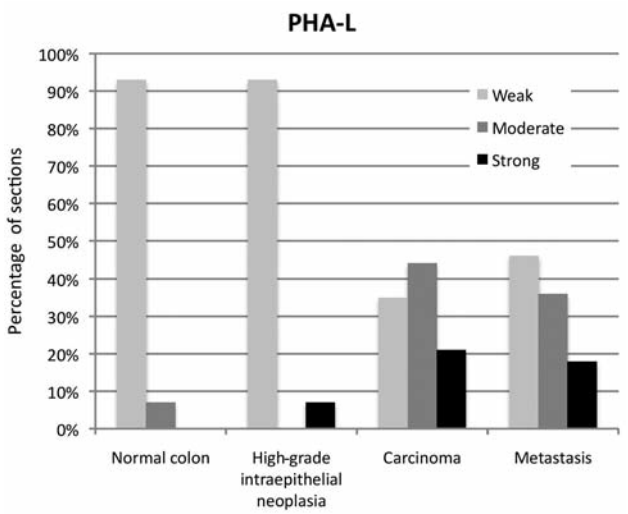

Figure 2. *Binding of Phaseolus vulgaris leukoagglutinin (PHA-L) to normal colon, high-grade intraepithelial neoplasia, carcinoma and metastasis by staining frequency.*

strong intensity and more than normal cells. In carcinomas, the brush border labeled very strongly; in metastases, the brush border was labeled at the same intensity as in normal enterocytes. No other membranes stained, the connective tissue did not stain or was only weakly labeled.

In the case of sections that did bind HPA (Figure 3), the healthy colon (Figure 4A) and the high-grade intraepithelial neoplasia tissues (Figure 4B) mostly weakly bound this lectin. A total of 64% of the colonic and 50% of the carcinoma *in situ* sections exhibited a stained brush border, the rest of the membrane was consistently unstained. The goblet cells remained unstained and the connective tissue exhibited little or no HPA binding. In normal colonic epithelium and high-grade intraepithelial neoplasia, the centrally arranged granules in the epithelial cells were strongly stained. Carcinomas (Figure 4C) and metastases (Figure 4D) mostly moderately bound this lectin, in some cases even strongly so. In carcinomas, the brush border bound to HPA in 56% of the cases, in metastases only in 32%. The rest of the cell membrane was not labeled. The connective tissue showed no or a little HPA binding.

DBA binding was very weak: all cells, ranging from normal enterocytes to metastatic ones, weakly bound DBA. Colon and high-grade intraepithelial neoplasia mostly exhibited a positive but weak signal at the brush border, while the basolateral membrane did not bind DBA. The goblet cells were stained in some cases. The connective tissue remained unstained. Carcinomas and metastases exhibited a mostly negative apical signal, the rest of the membrane and the connective tissue also remained unstained.

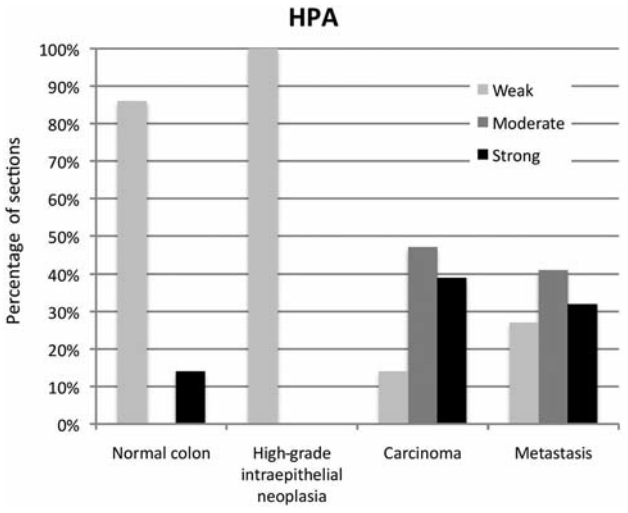

Figure 3. *Binding of Helix pomatia agglutinin (HPA) to normal colon, high grade intraepithelial neoplasia, carcinoma and metastasis by staining frequency.*

Con A was bound in nearly all cells in normal colon, high-grade intraepithelial neoplasia, carcinomas and metastases; the connective tissue was also stained. The goblet cells in colon and high-grade intraepithelial neoplasia sections were unstained.

Normal enterocytes exhibited mostly (50%) moderate GNA binding, while high-grade intraepithelial neoplasia mostly exhibited strong binding (86%). In normal colon and

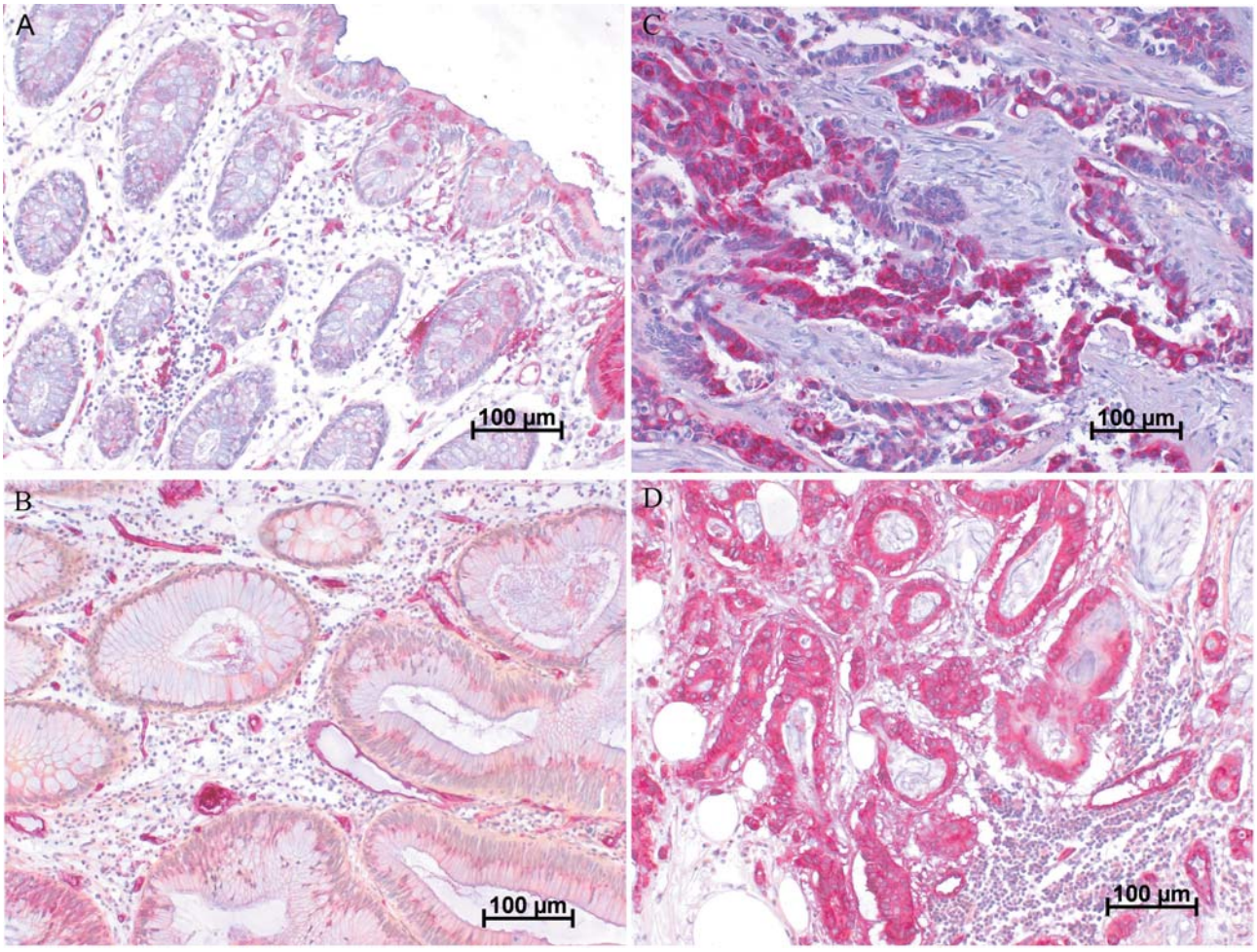

Figure 4. *Helix pomatia agglutinin binding to normal colon (A), high-grade intraepithelial neoplasia (B), carcinoma (C) and metastasis (D).*

high-grade intraepithelial neoplasia, the brush border was generally unstained in most cases. The majority (70%) of carcinoma cells exhibited strong binding to GNA. In 86% of the carcinoma cases, the brush border was labeled, in only 14% of the cases was the basal membrane stained. The connective tissue stained moderately or in parts strongly. Cancer cells in the metastases exhibited moderate GNA binding (59%). In 60% of the cases, the brush border was stained, while the rest of the cell membranes did not stain. In most cases, the connective tissue exhibited moderate GNA binding.

All sections, no matter if normal enterocytes or carcinoma cells, exhibited strong binding of SNA-I. In colon, the brush border mostly remained unstained, as did the rest of the membrane. In high-grade intraepithelial neoplasia, the majority of cells presented a positive SNA-I signal at the brush border, while the rest of the membrane remained unstained. The goblet cells and the high-grade intraepithelial neoplasia cells did not react with SNA-I. The connective tissue was always colored. In carcinomas and metastases, the brush border was always stained, as well as the connective tissue.

UEA-I bound to the majority of the epithelia of normal colon (100%) and high-grade intraepithelial neoplasia (93%) weakly. Connective tissue and the goblet cells remained unstained. Only 37% of the carcinoma sections presented moderate UEA-I binding. A total of 56% of the sections had a positive signal at the brush border, while the rest of the membrane was unstained. UEA-I mostly bound to cells in metastases weakly; 32% of the sections of metastases showed apical reactivity at the brush border, the rest of the membrane remained unreactive. In carcinomas and metastases, the connective tissue mostly remained unreactive.

Discussion

The aim of the present study was to investigate whether the binding of lectins with different carbohydrate specificities can detect changes in glycosylation associated with the malignant progression of colorectal cancer. The results show that some of the eight lectins, but not all, can be regarded as indicators of these changes. While binding of WGA, HPA, PHA-L, GNA, and in part UEA-I, indicated changes during malignant progression, SNA-I, Con A and DBA did not differ in their binding pattern during the different stages of malignant transformation.

It is significant that in the case of WGA, HPA and PHA-L, the percentage of cancer cells that bound these lectins was much higher for carcinomas and metastases than for normal colorectal tissue and for high-grade intraepithelial neoplasia, indicating a detectable change in glycosylation during malignant progression. These three lectins, with different carbohydrate specificities, stand out for their ability to decorate cells differently during malignant progression: WGA binds to *N*-acetylglucosamine and sialic acid (24), HPA binds to *N*-acetylgalactosamine, *N*-acetylglucosamine and sialic acid (25), and PHA-L is specific for β-1,6-branched oligosaccharides (26, 12); all have been associated with carbohydrate structures which are altered during malignant progression. Similar results were found by Boland *et al*., who detected changes in the mucus glycosylation of cancer cells using different lectins (27). The results for the binding pattern of HPA confirm those of previous studies stating that this lectin can serve as a good prognostic indicator in colorectal cancer (8, 12). It is remarkable that in an earlier study, colorectal carcinomas, like metastases, had a high tendency to bind HPA (8) and that the present study reveals the same finding. The results show that more than 10% of colonic sections bound to HPA – this can be explained by the fact that HPA binds to *N*-acetylgalactosamine on the surface of erythrocytes of blood group A. Although *N*-acetylgalactosamines are part of glycolipids, glycosaminoglycans and glycoproteins, only the *N*-acetylgalactosamines, being a component of glycoproteins, can be seen as a sugar residue playing an important role for metastasis as was demonstrated earlier (26, 28, 29). As described above, it has been hypothesized that this sugar residue recognized by HPA is a ligand for P- and E-selectins (13). These selectins function physiologically as mediators for adhesion of leukocytes to endothelial cells and for their extravasion through the endothelium from the vessels finally into organs (13, 30) – therefore indicating that tumor cells may use the same mechanism to enter another organ as leukocytes do (13). These data might possibly explain why HPA-positive tumor cells manage to metastasize and others do not. Similar hypotheses could be made for other carbohydrate structures that were found to be hypothetically part of the metastatic cascade in this study, such as fucose, as it is also part of the Lewis blood group antigens which serve as ligands for the two selectins (13). It can be speculated that these altered sugar residues serve as a ligands for specific selectins and therefore are responsible for the tumor becoming metastatic, with consequent poor prognosis.

Changes in the glycosylation of glycoproteins such as cadherin (31-34), integrin (35-37) and laminin (35), as well as the epithelial cell adhesion molecule EpCAM (38), were also found to have an impact on tumor progression. All these glycoproteins have specific *N*-glycosylation on their surface and take part in cell–cell adhesion, proliferation, cell migration, cell formation, and mechanical integrity, as well as in barrier functions. Since many types of cancer are directly associated with changes in cell–cell adhesion and tissue formation, glycan modification may have a considerable influence on cancer cell behavior (39). Previous studies revealed that modifications in glycoprotein *N*-glycosylation have an influence on malignant progression: the increased sialylation of β1-integrin was found to be involved in the migration of human colon cancer cells (40), the *N*-glycosylation of E-cadherin was discovered to be important for tumor development in breast cancer (33). Similar results are reported for melanoma (41). Almaraz *et al*. describe the modification of sialylated *N*-glycans because of metabolic flux and hypothesize that these changes enhance the metastatic potential of cancer cells (42). They identified that not all but some individual glycoproteins are altered by metabolic flux through sialic acid pathways; these glycoproteins are CD44 and integrin α6 (42). Since CD44 binds to selectins and since changed sialylation of CD44 enhances that binding, as the authors demonstrated, these data support the findings of Sperandio *et al*. (30) and Köhler *et al*. (13), as described before. Furthermore, modifications of *N*-linked glycosylation on the surface of integrin α5β1 have an impact on the adhesion of monocytes to the vascular endothelium and subsequently on their transmigration through the vascular enothelium (43). Changes in the glycosylation of integrin α5β1 can be due to the activity of *N*-acetylglucosaminyltransferase V. All in all, it can be assumed that cancer cells make use of a similar mechanism during their metastatic pathway. The increased binding, especially of WGA, HPA and PHA-L, in our study during malignant progression might also underline the altered *N*-glycosylation of the previously discussed glycoproteins. Glycoprotein functions such as in cell migration and cell–cell adhesion and their altered glycosylation in cancer can explain many tumor characteristics, such as the ability to metastasize, the progression of dedifferentiation, the loss of the physiological tissue structure, or the deranged location of cells to one another. Since most changes of lectin binding were seen in carcinomas, one can presume that metastatic competence is secured late in the course of the biology of this disease.

Our findings may also have practical implications. As lectins bind to carbohydrate residues on the cell surface, appropriately labeled lectins may serve as probes in endoscopy. Due to the fact that there are still adenomas that remain undetected by the colonoscopist (44), with a polyp error rate of 22% (45) or even higher (46), it has been suggested that lectins could be used for solving this problem. A possible scenario could be that patients drink a solution containing labeled lectins before their colonoscopy; the lectins would preferentially bind to adenomas in contrast to normal colorectal tissue and could consequently help the colonoscopist to find these altered structures. Some research has already identified WGA as a biomarker for endoscopic visualization of Barrett's esophagus to detect dysplastic esophageal tissue (47). Nevertheless, this idea presupposes that there is a noticeable difference between the binding pattern of the lectin to the brush border of normal and neoplastic tissue – which is applicable to SNA-I: as the brush border of the colon sections was not colored but the brush border of high-grade intraepithelial neoplasia, carcinoma and metastases gave a positive signal, this lectin could possibly serve as a marker in colonoscopy.

It can be concluded that binding of lectins specific for mannose, *N*-acetylgalactosamine, *N*-acetylglucosamine, sialic acid, β-1,6-branched oligosaccharides and α-1-fucose can be associated with malignant progression in colorectal cancer. Nevertheless, in order to decode the complete metastatic cascade and the entire metastatic mechanism, much more research is needed.

Acknowledgements

The Authors would like to thank Jennifer Schröder-Schwarz and Tobias Gosau who all work as technicians at the Institut für Anatomie Experimentelle Morphologie, Universitätsklinikum Eppendorf, Hamburg, Germany, for their help in histochemical procedures and treatments.

References

1 Robert-Koch-Institut: Kapitel "Krebs gesamt" aus dem Beitrag zur Gesundheitsberichterstattung des Bundes "Verbreitung von Krebserkrankungen in Deutschland", date of release 23.02.2010.

2 Cho KR and Vogelstein B: Genetic alterations in the adenoma–carcinoma sequence. Cancer 7*0(6 Suppl)*: 1727-1731, 1992.

3 Sugarbaker EV and Ketcham AS: Mechanisms and Prevention of Cancer dissemination: an overview. Seminars in Oncology 4, 19, 1977.

4 Saegesser M: Aktuelle Probleme in Chirurgie und Orthopädie – Band 14, Metastasen, released by C.Burri, Ch. Herfarth, M. Jäger, Verlag Hans Huber Bern, 1980.

5 Pecorino L: Molecular biology of cancer, Oxford University Press, Oxford, United Kingdom, 2005.

6 Brooks SA, Dwek MV and Schumacher U: Functional and Molecular Glycobiology, BIOS Scientific Publishers, Oxford, United Kingdom, 2002.

7 Gabius HJ and Gabius S: Lectins and Cancer, Springer, 1.Auflage, Berlin, Germany, 1991.

8 Schumacher U, Higgs D, Loizidou M, Pickering R, Leathem AJ and Taylor I: *Helix pomatia* agglutinin binding is a useful prognostic indicator in colorectal carcinoma, Cancer *74(12)*: 3104-3107, 1994.

9 Kakeji Y, Tsujitani S, Mori M, Maehara Y and Sugimachi K: *Helix pomatia* agglutinin binding activity is a predictor of survival time for patients with gastric carcinoma. Cancer *68(11)*: 2438-2442, 1991.

10 Leathem AJ and Brooks SA: Predictive value of the lectin binding on breast-cancer recurrence and survival. Lancet *1(8541)*: 1054-1056, 1987.

11 Thies A, Moll I, Berger J and Schumacher U: Lectin binding to cutaneous malignant melanoma: HPA is associated with metastasis formation. Br J Cancer *84(6)*: 819-823, 2001.

12 Schumacher U and Adam E: Lectin histochemical HPA-binding pattern of human breast and colon cancers is associated with metastases formation in severe combined immunodeficient mice. Histochem J *29(9)*: 677-684, 1997.

13 Köhler S, Ullrich S, Richter U and Schumacher U: E-/P-selectins and colon carcinoma metastasis: first *in vivo* evidence for their crucial role in a clinically relevant model of spontaneous metastasis formation in the lung. Br J Cancer *102(3)*: 602-609, 2009.

14 Fernandes B, Sagman U, Auger M, Demetrio M and Dennis JW: Beta 1-6 branched oligosaccharides as a marker of tumor progression in human breast and colon neoplasia. Cancer Res *51(2)*: 718-723, 1991.

15 Lange T, Ullrich S, Müller I, Nentwich MF, Stübke K, Feldhaus S, Knies C, Hellwinkel OJ, Vessella RL, Abramjuk C, Anders M, Schröder-Schwarz J, Schlomm T, Huland H, Sauter G and Schumacher U: Human Prostate cancer in a clinically relevant xenograft mouse model: identification of β(1,6)-branched oligosaccharides as a marker of tumor progression. Clin Cancer Res *18(5)*: 1364-1373, 2012.

16 Mitchell BS, Brooks SA, Leathem AJ and Schumacher U: Do HPA and PHA-L have the same binding pattern in metastasizing human breast and colon cancers? Cancer Lett *123(1)*: 113-119, 1998.

17 Iwakawa K, Ueda N, Murao S and Kobayashi N: Altered carbohydrate composition in colorectal adenomas and carcinomas: histochemical characterization of *N*-acetylgalacto-samine, L-fucose, and o-acetylated sialic acid. J Gastroenterol *31(1)*: 24-32, 1996.

18 Lazaris AC, Chatzigianni EB, Paraskevakou H, Tseleni-Balafouta S and Davaris PS: Lectin histochemistry as a predictor of dysplasia grade in colorectal adenoma. Pathol Oncol Res *6(4)*: 265-71, 2000.

19 Caldero J, Campo E, Ascaso C, Ramos J,Panades MJ and Rene JM: Regional distribution of glycoconjugates in normal, transitional and neoplastic human colonic mucosa. A histochemical study using lectins. Virchows Arch A Pathol Anat Histopathol *415(4)*: 347-56, 1989.

20 Bresalier RS, Boland CR and Kim YS: Regional differences in normal and cancer-associated glycoconjugates of the human colon. J Natl Cancer Inst *75(2)*: 249-260, 1985.

21 Schnegelsberg B, Schumacher U and Valentiner U: Lectin histochemistry of metastasizing and non-metastasizing breast and colon cancer cells. Anticancer Res *31(5)*: 1589-1597, 2011.

22 Seelentag WK, Li WP, Schmitz SF, Metzger U, Aeberhard P, Heitz PU and Roth J: Prognostic value of beta1,6-branched oligosaccharides in human colorectal carcinoma. Cancer Res *58(23)*: 5559-5564, 1998.
23 Naish S: Handbuch immunhistochemischer Färbemethoden II, Dako Corporation, Carpinteria, Kalifornien, USA, 1989.
24 Crouzier T, Beckwitt CH and Ribbeck K: Mucin multilayers assembled through sugar-lectin interactions. Biomacromolecules *13(10)*: 3401-8, 2012.
25 Rambaruth ND, Greenwell P and Dwek MV: The lectin *Helix pomatia* agglutinin recognizes O-GlcNAc containing glycoproteins in human breast cancer. Glycobiology *22(6)*: 839-848, 2012.
26 Brooks SA: The involvement of *Helix pomatia* lectin (HPA) binding *N*-acetylgalactosamine glycans in cancer progression. Histol Histopathol *15(1)*: 143-158, 2000.
27 Boland CR, Montgomery CK and Kim YS: Alterations in human colonic mucin occuring with cellular differentiation and malignant transformation. Proc Natl Acad Sci USA *79(6)*: 2051-2055, 1982.
28 Schumacher U, Mitchell BS, Brooks SA, Delpech B and Leathem AJ: Does the lectin *Helix pomatia* agglutinin bind to hyaluronic acid in breast and colon cancer? Acta Histochem *98(4)*: 435-440, 1996.
29 Schumacher U, Adam E, Brooks SA and Leathem AJ: Lectin-binding properties of human breast cancer cell lines and human milk with particular reference to *Helix pomatia* agglutinin, J Histochem Cytochem *43(3)*: 275-281, 1995.
30 Sperandio M: Selectins and glycosyltransferases in leukocyte rolling *in vivo*, FEBS J *273(19)*: 4377-4389, 2006.
31 Pinho SS, Seruca R, Gärtner F, Yamaguchi Y, Gu J, Taniguchi N and Reis CA: Modulation of E-cadherin function and dysfunction by *N*-glycosylation, Cell Mol Life Sci *68(6)*: 1011-1020, 2011.
32 Guo HB, Johnson H, Randolph M and Pierce M: Regulation of homotypic cell–cell adhesion by branched *N*-glycosylation of *N*-cadherin extracellular EC2 and EC3 domains. J Biol Chem *284(50)*: 34986-34997, 2009.
33 Pinho SS, Osório H, Nita-Lazar M, Gomes J, Lopes C, Gärtner F and Reis CA: Role of E-cadherin *N*-glycosylation profile in a mammary tumor model. Biochem Biophys Res Commun *379(4)*: 1091-1096, 2009.
34 Vagin O, Tokhtaeva E, Yakubov I, Shevchenko E and Sachs G: Inverse correlation between the extent of *N*-glycans branching and intercellular adhesion in epithelia. J Biol Chem *283(4)*: 2192-2202, 2008.
35 Gu J, Isaji T, Xu Q, Kariya Y, Gu W, Fukuda T and Du Y: Potential roles of *N*-glycosylation in cell adhesion. Glycoconj J *29(8-9)*: 599-607, 2012.
36 Kariya Y and Gu J: *N*-Glycosylation of β4 integrin controls the adhesion and motility of keratinocytes. PLoS One *6(11)*: e27084, 2011.
37 Janik ME, Litynska A and Vereecken P: Cell migration – the role of integrin glycosylation, Biochim Biophys Acta *1800(6)*: 545-555, 2010.
38 Munz M, Fellinger K, Hofmann T, Schmitt B and Gires O: Glycosylation is crucial for stability of tumor and cancer stem cell antigen EpCAM, Front Biosci *13*: 5195-5201, 2008.
39 Langer MD, Guo H, Shashikanth N, Pierce JM and Leckband DE: *N*-Glycosylation alters cadherin-mediated intercellular binding kinetics, J Cell Sci *125(Pt 10)*: 2478-2485, 2012.
40 Lee M, Lee HJ, Seo WD, Park KH and Lee YS: Sialylation of integrin β1 integrin is involved in radiation-induced adhesion and migration in human colon cancer cells, Int J Radiat Oncol Biol Phys *76(5)*: 1528-1536, 2010.
41 Krähling H, Mally S, Eble JA, Noël J, Schwab A and Stock C: The glycocalyx maintains cell surface pH nanoenviroment crucial for integrin-mediated migration of human melanoma cells, Pflugers Arch *458(6)*: 1069-1083, 2009.
42 Almaraz RT, Tian Y, Bhattarcharya R, Tan E, Chen SH, Dallas MR, Chen L, Zhang Z, Zhang H and Konstantopoulos K: Metabolic Flux increases glycoprotein sialylation implications for cell adhesion and cancer metastasis. Mol Cell Proteomics *11(7)*: M112.017558, 2012.
43 Yang HM, Yu C and Yang Z: *N*-Acetylglucosaminyltransferase V negatively regulates integrin α5β1-mediated monocyte adhesion and transmigration through vascular endothelium, Int J Oncol *41(2)*: 589-598, 2012.
44 Lieberman D: A call to action – measuring the quality of colonoscopy. N Engl J Med *355(24)*: 2588-2589, 2006.
45 Van Rijn JC, Reitsma JB, Stoker J, Bossuyt PM, van Deventer SJ and Dekker E: Polyp miss rate determined by tandem colonoscopy: a systematic review. Am J Gastroenterology *101(2)*: 343-350, 2006.
46 Heresbach D, Barrioz T, Lapalus MG, Coumaros D, Bauret P, Potier P, Sautereau D, Boustière C, Grimaud JC, Barthélémy C, Sée J, Serraj I, D'Halluin PN, Branger B and Ponchon T: Miss rate for colorectal neoplastic polyps: a prospective multicenter study of back-to-back video colonoscopies, Endoscopy *40(4)*: 284-290, 2008.
47 Bird-Liebermann EL, Neves AA, Lao-Sirieix P, O'Donovan M, Novelli M, Lovat LB, Eng WS, Mahal LK, Brindle KM and Fitzgerald RC: Molecular imaging using fluorescent lectins permits rapid endoscopic identification of dysplasia in Barrett's esophagus, Nat Med *18(2)*: 315-321, 2012.

Received May 30, 2015
Revised July 9, 2015
Accepted July 13, 2015