Decreased Expression of Claudin 1, 3, 4, 5 and 7: A New Prognostic Marker in Colon Carcinoma

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Abstract

Background and objective: Claudins are members of a large family of tissue specific adherent proteins which have been suggested as tumor markers. In this study we analyzed the protein expression of claudin 1, 3, 4, 5 and 7, their clinical significance and association with tumor growth pattern in colon carcinoma.

Methods: Immunohistochemical staining (IHC) was used to detect the expression of claudin 1, 3, 4, 5 and 7 in samples diagnosed with colon carcinoma as well as the adjacent normal mucosa. Complexity index (CI) was calculated using images of cytokeratin-8 stained slides of the tumours using Photoshop CS, Fovea Pro, and Image J computer programs. The results from the IHC and CI were correlated to clinicopathological parameters as well as 5-years survival of the patients diagnosed with colon carcinoma.

Results: Significantly high staining intensity was observed in normal mucosa as compared to colon cancer tissue for claudin 4 (p=0.031) and 7 (p=0.011) and number of stained cells for claudin 4 (p=0.001). A significant association was also observed between weaker expression at the invasive front of the tumor tissue and heterogenous expression of claudin 1 (p=0.000), claudin 3(p=0.003), claudin 5(p=0.001) and claudin 7(p=0.000). There was no significant correlation between the expression of claudins, CI and clinicopathological parameters. Similarly, no significant association was found between claudin expression and 5-year survival of the patients diagnosed with colon carcinoma.

Conclusion: Altered expression of claudin 1, 3, 4, 5 and 7 in colon carcinoma cells may play a promoting role in colon carcinoma development and is inversely proportional to higher expression at the invasive front of the tumor. Claudin protein expression can be used as a tumor marker since the expression generally is weaker in tumor tissue compared to the normal mucosa.

Keywords: Tight junctions; Complexity index; Immunohistochemistry; Heterogenous expression

Introduction

Intestinal epithelium cells are joined together to form tight junction (TJ) complexes which control the paracellular diffusion between the cells. These complexes play an important role in maintaining the concentration differences of small molecules across epithelial cell sheets by sealing the plasma membranes of adjacent cells at the apical surface. This results in the creation of a continuous impermeable or semi permeable barrier for diffusion across the cell sheets [1-3].

The claudin (CLDN) name originates from the Latin word "claudere" ("to close"), which indicates the barrier role of those proteins. This family of proteins consists of 27 known claudin members with a size ranging from 20-27 kDa [4,5]. They are essential for the formation of tight junctions between epithelial cells together with other adherent proteins called occludins [6]. Several studies have reported altered expression of Claudins as prognostic marker in various human cancers such as tumors of the gastrointestinal tract, renal, ovary, pancreas, breast, melanoma, liver, lung, uterus and prostate [6,7].

A study by Bello et al. shows up regulation of CLDN 3 and CLDN 4 in ovarian cancer as well as high expression of claudin 7 as an early event in carcinoma of the tongue [8]. Furthermore, CLDN 1 has also been found to be down regulated in colorectal carcinoma suggesting a probable target of beta catenin/Tcf signalling [1]. According to previous studies, some reports have presented decreased expression of claudin 5 in hepatocellular and renal carcinomas [8-10].

There are relatively few reports on the protein expression of claudins in colon carcinoma and most of the studies have determined the expression of one or two claudins [11-13]. We aimed this study to evaluate the expression and significance of claudin 1, 3, 4, 5 and 7 proteins in paired normal and tumor tissue samples from 61 patients diagnosed with colon carcinoma and to uncover any possible association between claudin expression and growth pattern of tumor.

To analyze a tumor, its size and growth pattern are important variables as tumor progression in surrounding tissues shows different patterns depending upon the, invasive margin, number and distribution of tumor cells. Infiltrative and expensive are two different categories of tumor growth in which former has irregular and coarse invasive front and considered as responsible for worst prognosis [14,15]. Multiple scoring systems have been introduced to describe the tumor growth in different carcinomas [16]. In 2008, a computer software based technique was introduced by Franzen and Hahn-Strömberg in which the tumor invasive front was graded quantitatively from 1-5, called complexity index (CI) where 1 represents the smooth and regular invasive front.
while tumors with 5 CI score have highly irregular invasive front with separated tumor cells and clusters [17]. This classification was based upon the fractal dimensions, and number of tumor cells. The proteins, which are involved in the intercellular adhesions are important in maintaining the morphology of the tumor and affect the invasion and metastasis [18,19]. Being a part of tight junction complex, claudins have a significant role in cell adhesion [20]. So we hypothesize that there is a correlation between tumor progression, tumor growth pattern and adhesive protein expression.

The aim of our study was to compare the expression patterns of 5 different claudin proteins (claudin 1,3,4,5 and 7) in normal and paired human colon carcinoma tissue samples and to correlate these results with 5-year survival data of the patients, growth pattern of tumor and clinicopathological parameters of the patients diagnosed with colon carcinoma.

Material and Methods

Sample selection

From the pathology archives, 61 samples diagnosed with colon carcinoma from 2000 to 2009 at Örebro University Hospital were randomly selected. The age of the selected patients at diagnosis varied from 51 to 90 years for both men and women. Equal numbers of control samples were selected from the same patients to compare with tumor samples. Rectal carcinoma samples were excluded since these are often treated with radiotherapy prior to surgery which may result in altered morphologic characteristics. Uppsala University Ethical Committee, Uppsala, Sweden, approved this study.

Immunohistochemistry (IHC) staining

4 μm thick sections were cut from formalin fixed paraffin-embedded tissue (FFPE) blocks using a Leica microtome (Buffalo Grove, USA) and the sections were placed on super-frost slides and incubated for 60 min at 62°C. Sections were pretreated for 1 hour and 20 min with EDTA buffer saline solution pH of 9.3 at 97°C.

Primary antibodies anti-Claudin 1 and anti-Claudin 7 were purchased from Invitrogen (California, USA) and anti-Claudin-3,-4, and 5 from Abcam (Cambridge, UK). All antibodies were polyclonal rabbit primary antibodies designed specifically for IHC of FFPE tissue. Immunohistochemistry staining was performed according to manufacturer’s protocol (Dako cytomation) with incubation of the primary antibodies for 30 minutes at room temperature with working dilutions of 1:200, 1:400, 1:1200 and 1:12000 for anti-claudin 1, 3, 4, 5 and 7 respectively. Staining was performed using an Autostainer (Dako Produktionssvej, Glostrup, Denmark).

Scoring of slides

Slides were scored in accordance with earlier publications on intensity of staining as well as number of stained cells, with intensity scores of 0, 1, 2 and 3 where 0=no staining, 1=weak, 2=moderate and 3=strong staining. The number of stained cells was scored accordingly with grade <10%, grade 1=10-50%, grade 2=50-80% and grade 3=80% stained cells.

Complexity index

40 samples from the patients diagnosed with colon carcinoma were selected randomly for computer image analysis from the same samples used for claudin expression study. Sectioning, staining, and image processing was performed using the same method described by Franzen et al. [1,17]. In short, images from the invasive front of the tumor area were taken by using a Leica DC200 digital camera mounted on a Leica DMRXE microscope with a 10X objective lens (Leica Microsystems GmbH, Wetzlar, Germany). From each specimen on average of 8 (4-12) images were captured for every tumor specimen. The number of images depended upon the length of the tumor-stromal area. Images were thresholded into white and black where black was the tumor area with white background. The black area was then removed so that only the outline of the tumor was left. The number of tumor cells, tumor cell clusters and fractal dimensions were calculated using the tumor outline image. The results were translated into a CI value ranging from 1-5.

Statistical analysis

Continuous variables were measured by mean and standard deviation and categorical variables by frequencies. Significance of association between colon carcinoma and adjacent normal tissue claudin proteins expression was measured statistically by McNemar test. To determine any statistical association between expression of claudins in colon cancer tissue and possible explanatory variable, Pearson’s Chi-squared and Fisher exact test were applied appropriately. To determine a correlation between complexity index, claudin expression and clinicopathological parameters, Spearman’s correlation coefficient (Rho) test was used. Pearson’s Chi-squared test was used to observe heterogeneity in expression. Kaplan Meier test was performed to determine any association between claudin expression and 5-years survival of the patients. Two-sided p-value of ≤0.05 was taken as significant. SPSS version 20 (SPSS Inc., Chicago, IL, USA) and Stata (version SE11.1) were used for statistical analysis.

Results

Clinicopathologic study

To determine the protein expression of claudin 1, 3, 4, 5 and 7 in colon cancer and in adjacent normal mucosa tissue samples, a random selection of 61 patients’ samples were analyzed using immunohistochemistry. The age of the patients ranged from 51 to 90 years. There were 34 samples from men and 27 from women. 34 of the tumor samples were from right colon and 27 from the left side of colon. Different clinical and pathological parameters (gender, tumor wall penetration (T), lymph noded metastasis (N), distant metastasis (M), Dukes stages, differentiation and localisation of the patient tumors) are provided in Table 1.

Immunohistochemical study

Differential expression of claudin 1, 3, 4, 5 and 7 between normal, highly differentiated and moderately differentiated tumor tissue cells are shown in Figure 1.

Both the tumor and adjacent normal colonic mucosa were separately scored. The normal colonic mucosa showed high staining intensity for all of these five claudins. The numbers of samples with cytoplasmic claudins staining were very low, only two cases showed protein expression for claudins 4, 7 and one case for claudin 3 and 5. All samples with cytoplasmic expression were from tumor tissue and were at T4 stage.

All the studied claudin proteins show moderate to high staining intensity for normal as well as tumor samples (Table 2), however there was a significant difference in staining intensity between tumor and normal tissues for claudin 4 (p<0.031) and 7 (p=0.011).

Staining intensities of claudins 1, 3, 4, 5 and 7 expressions in tumor tissues were also compared with different clinical and pathological variables (Table 1). For the tumor wall penetration, proportion of
tumors with lower claudin 1, 4, 5 and 7 expressions were higher at T3+T4 stages then with higher expression, but claudin 3 expression was observed reverse. In case of lymph node metastasis, variable expression was observed for different claudins such as, for claudin 3, 4 and 5 proportion of tumor with lower expression and lymph node metastasis was comparatively higher to increased expression and lymph node metastasis, but opposite results were found for claudin 1 and 7. Similar results were observed between claudins expression and other clinicopathological parameters such distant metastasis, Duke Stages and differentiation. However, in either case, none of the parameter was significantly associated with either claudin that we determined in current project. Though, a trend was observed between claudin 3 expressions and medium tumor differentiation (p=0.087), and also between claudin 5 and lymph node metastasis (p=0.088).

We also compared the number of stained cells between normal and tumor samples. Only claudin 4 has significantly low number of stained cells in tumor samples as compared with normal samples (p=0.001), while an opposite trend was observed for claudin 5 (p=0.052). No significant association could be recognized for number of stained cells between tumor and normal tissues in claudin 1, 3 and 7 (p>0.05) (results not shown).

Additionally, claudin 1, 3, 4, 5 and 7 were further studied for heterogeneous expression, weak claudin expression at invasive border and cell membranes of the tumor. Heterogeneity of claudin expression was compared with clinicopathological parameters of the patients (TNM, tumor differentiation), weaker expression of claudins at membrane and invasive front of tumor to find any significant association. Heterogeneity in expression was significantly associated with weak expression at invasive border of tumor for claudin1 (p<0.001), claudin 3 (p=0.003), claudin 4 (p=0.026), claudin 5 (p=0.001) and claudin 7 (p<0.001) (Table 3).

Claudins and complexity index

We also analyzed the CI value of each specimen with expression of claudin proteins (claudin 1,3,4,5 and 7) and clinicopathological parameters (including tumor wall penetration (T), lymph node metastasis (N), distant metastasis (M) and differentiation) of the patients. Results indicate a trend for significant association between claudin 5 expression and CI (p=0.06). Similar results were seen within tumor wall penetration and high value of CI (p=0.08). CI was not significantly associated with other studied claudin expression or clinicopathological parameters of the patients. Results are shown in Table 4.

To investigate any possible association between claudin expression (claudin 1, 3, 4, 5 and 7) and 5-years survival of the patients, we

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**Table 1:** Clinical and pathological characteristics of the patient data.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
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</tr>
<tr>
<td>Male</td>
<td>34 (56%)</td>
</tr>
<tr>
<td>Female</td>
<td>27 (44%)</td>
</tr>
<tr>
<td>T Stages</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>T2</td>
<td>22 (36%)</td>
</tr>
<tr>
<td>T3</td>
<td>32 (52%)</td>
</tr>
<tr>
<td>T4</td>
<td>7 (11%)</td>
</tr>
<tr>
<td>LN metastasis</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>38 (63%)</td>
</tr>
<tr>
<td>N1</td>
<td>10 (16%)</td>
</tr>
<tr>
<td>N2</td>
<td>13 (21%)</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>17 (27.8%)</td>
</tr>
<tr>
<td>M1</td>
<td>38 (62.3%)</td>
</tr>
<tr>
<td>M2</td>
<td>6 (9.8%)</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>Med</td>
<td>37 (60%)</td>
</tr>
<tr>
<td>Low</td>
<td>18 (30%)</td>
</tr>
<tr>
<td>Dukes</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>14 (23%)</td>
</tr>
<tr>
<td>B</td>
<td>23 (38%)</td>
</tr>
<tr>
<td>C+D</td>
<td>24 (39%)</td>
</tr>
<tr>
<td>Localisation</td>
<td></td>
</tr>
<tr>
<td>Right Colon</td>
<td>34 (56%)</td>
</tr>
<tr>
<td>Left Colon</td>
<td>27 (44%)</td>
</tr>
<tr>
<td>LN metastasis: Lymph node metastasis</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 2:** Claudin expression in normal vs tumor samples from the patients diagnosed with colon carcinoma.

<table>
<thead>
<tr>
<th>Claudin</th>
<th>Type of tissue</th>
<th>Number of stained cells High (N)</th>
<th>Medium (N)</th>
<th>p-value</th>
<th>Intensity of staining High (N)</th>
<th>Medium (N)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin 1</td>
<td>Tumor</td>
<td>32</td>
<td>29</td>
<td>0.131</td>
<td>25</td>
<td>36</td>
<td>0.582</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>59</td>
<td>2</td>
<td></td>
<td>28</td>
<td>33</td>
<td>0.803</td>
</tr>
<tr>
<td>Claudin 3</td>
<td>Tumor</td>
<td>37</td>
<td>24</td>
<td>0.963</td>
<td>31</td>
<td>30</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>51</td>
<td>10</td>
<td></td>
<td>53</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Claudin 4</td>
<td>Tumor</td>
<td>47</td>
<td>14</td>
<td>0.001</td>
<td>35</td>
<td>35</td>
<td>0.739</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>52</td>
<td>9</td>
<td></td>
<td>46</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Claudin 5</td>
<td>Tumor</td>
<td>49</td>
<td>12</td>
<td>0.052</td>
<td>35</td>
<td>26</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>40</td>
<td>21</td>
<td></td>
<td>58</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Claudin 7</td>
<td>Tumor</td>
<td>55</td>
<td>6</td>
<td>0.635</td>
<td>39</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>59</td>
<td>2</td>
<td></td>
<td>55</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

High=High expression, Medium= Medium expression, N= Number of samples

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**Figure 1:** IHC staining for claudin 1 (A), claudin 3 (B), Claudin 4(C), Claudin 5 (D), Claudin 7(E) and Normal mucosa (N). Intensity score strong can be seen in A1-E1 and intensity score moderate can be seen in A2-E2.
Discussion

Tight junctions (TJs) are apical intercellular junctions in epithelial and endothelial cells. The two major functions defined for tight junctions are regulation of paracellular permeability and maintenance of the cell polarity through their barrier functions. Claudins are among the proteins, which form tight junctions, assist as barrier protein and regulate the permeability of blood vessels and epithelium in different types of tissues [21-24].

Considering the involvement of tight junction proteins in the regulation of epithelial proliferation and their potential usefulness as novel tools in cancer diagnosis, prognosis and treatment, we aim our study was to evaluate the expression and significance of claudin 1, 3, 4, 5 and 7 proteins in paired normal and tumor tissue samples from 61 patients diagnosed with colon carcinoma and correlate the expression with growth pattern of the tumor. To address this issue, immunohistochemistry was used to determine the patterns of distribution and intensity of expression of Claudins between normal and adjacent tumor cells.

In our study, we found that most of the normal tissue cells showed a strong claudin expression compared to the corresponding cancer tissue (Figure 1). Association between normal and paired cancer tissues was very high for claudin 4 and 7 (Figures B1 and E1). Significant difference was observed between tumor and normal tissue staining intensities for claudin 4 (p=0.031) and 7 (p=0.011) as there was high expression in normal samples as compared to tumors. Similar results were observed by Ersoz et al. in colorectal cancer and Jung et al. in gastric cancer [25,26]. Recently, Suren et al. reported a correlation between claudin 4 and aggressive behavior in colorectal carcinoma [27]. On the other hand, opposite results were found by Hwang et al. and De Oliveira et al. where claudin 4 was upregulated in gastric and colorectal cancer respectively [28,29]. However, the complete correlation between claudin 4 overexpression and invasive capacity of cancers have not been fully explained. Further studies have been warranted to explore the correlation between claudin 4 and various types of cancers.

Claudin 7 was studied by Komensky et al. in breast cancer and he reported low expression as compared with normal breast epithelium which is similar to our findings of claudin 7 in colon carcinoma [30]. Sauer et al. reported the reduced expression of Claudin 7 correlated with metastatic disease and higher tumor grade [31]. Similarly, low expression of claudin 7 was reported in prostate and oesophageal cancers [7]. Regarding claudin 1, 3 and 5, however, we did not find any significant association between expression intensities of (p>0.05).

We further explore the correlation between number of stained cells in tumor and normal samples. Claudin 4 (p=0.001) has significantly low number of stained cells in tumor samples as compared with normal samples but the opposite was seen for claudin 5 (p=0.052). No significant association could be recognized for number of stained cells between tumor and normal tissues in claudin 1, 3 and 7 (p>0.05). Previously, Caruso et al., and Bukho et al. reported the elevated levels of claudin 1 in colorectal cancer [32]. Similarly, claudin 7 was significantly associated with higher risk of distant metastasis in colorectal cancer [27,33].

Role of claudin family in tumors is controversial [4,34]. According to a study Seo et al., a decrease expression of claudin 1 is correlated to prostate carcinoma and was recommended as an important marker for lung adenocarcinoma [35,36]. Initially it was considered that claudin 5 is expressed only in endothelial cells, but later on it was found to

![Figure 2: Kaplan Meier survival curve showing relationship between claudin 1 expression and 5-year survival of patients diagnosed with colon carcinoma.](image)

<table>
<thead>
<tr>
<th>Claudin</th>
<th>Weak exp inv front</th>
<th>Weak memb exp</th>
<th>T</th>
<th>N</th>
<th>M</th>
<th>Differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin 1</td>
<td>0.000</td>
<td>0.845</td>
<td>0.453</td>
<td>0.73</td>
<td>0.953</td>
<td>0.635</td>
</tr>
<tr>
<td>Claudin 3</td>
<td>0.003</td>
<td>0.717</td>
<td>0.244</td>
<td>0.479</td>
<td>0.903</td>
<td>0.811</td>
</tr>
<tr>
<td>Claudin 4</td>
<td>0.026</td>
<td>0.905</td>
<td>0.244</td>
<td>0.479</td>
<td>0.903</td>
<td>0.398</td>
</tr>
<tr>
<td>Claudin 5</td>
<td>0.001</td>
<td>0.264</td>
<td>0.453</td>
<td>0.73</td>
<td>0.953</td>
<td>0.635</td>
</tr>
<tr>
<td>Claudin 7</td>
<td>0.000</td>
<td>0.462</td>
<td>0.453</td>
<td>0.73</td>
<td>0.953</td>
<td>0.635</td>
</tr>
</tbody>
</table>

Table 3: Correlation between heterogeneous claudin expression and clinicopathological data of the patients diagnosed with colon carcinoma.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Claudin 1</th>
<th>Claudin 3</th>
<th>Claudin 4</th>
<th>Claudin 5</th>
<th>Claudin 7</th>
<th>Claudin 1 -0.23</th>
<th>Claudin 3 -0.04</th>
<th>Claudin 4 0.26</th>
<th>Claudin 5 0.06</th>
<th>Claudin 7 -0.09</th>
<th>T 0.08</th>
<th>N -0.13</th>
<th>M 0.18</th>
<th>Differentiation -0.4</th>
</tr>
</thead>
</table>

Table 4: Correlation of CI with Claudin expression and clinicopathological parameters of the patients.

Kaplan-Meier analysis (Figure 2). No significant relationship was observed between claudin expression pattern and 5-years survival of the patients diagnosed with colon carcinoma. Statistical results for association between claudin expression and survival are p=0.612, p=0.358 p=0.533, p=0.846 and p=0.538 for claudin 1, claudin 3, claudin 4, claudin 5 and claudin 7 respectively (Table 5).

Table 5: Relationship between claudin expression and survival of the Patients diagnosed with colon carcinoma.
be expressed in various other cancer cell types as in hepatocellular and renal carcinomas, lower levels of claudins 4 and 5 have been determined [37]. Similarly, a decreased expression of claudin 3 has been associated with ovarian epithelial carcinoma [38,39]. The possible reason for discrepancies could be the tumors selected in different studies were at different stages of progression, location of the tumor within the organ and also evaluation of expression at different parts of tumor such as invasive front and centrally located tumor cells. In this study, rectal samples were excluded as they undergo radiation therapy which could possibly have effects on the final results. However, the molecular mechanism responsible for the differential expression of claudins in cancer still remains unclear. A much better knowledge of the particular functions of claudins, elucidation of mutations responsible for expression and regulation in cancers could provide the important information for future therapeutic interventions.

Intensities of claudin 1, 3, 4, 5 and 7 expressions in tumor tissues were also compared with different clinical and pathological variables. For the tumor wall penetration, proportion of tumors with lower claudin 1, 4, 5 and 7 expressions were higher at T3-T4 stages then with higher expression, but claudin 3 expression was observed reverse. In case of lymph node metastasis, variable expression was observed for different claudins such as, for claudin 3, 4 and 5 proportion of tumor with lower expression and lymph node metastasis was comparatively higher to increased expression and lymph node metastasis, but opposite results were found for claudin 1 and 7. Similar results were observed between Claudins expression and other clinicopathological parameters such distant metastasis, Duke stages and differentiation. However, in either case, none of the parameter was significantly associated with either claudin that we determined in current project. Though, a trend was observed between claudin 3 expressions and medium tumor differentiation (p=0.087), and also between claudin 5 and lymph node metastasis (p=0.088).

A 5-years survival analysis did not show any statistically significant association between claudin expression in patients diagnosed with colon carcinoma. A possible explanation for these results could be small sample size and different stages of colon carcinoma of selected patients in our study.

IHC expression of claudins was also examined to expose any possible association between heterogeneity of expression, weaker expression at cell membrane and invasive border of the tumor and clinicopathological parameters of the patients diagnosed with colon carcinoma. A significant correlation was observed between heterogeneity and weaker expression of claudins at invasive front of the tumor. This reveals the fact that when claudins are not homogenous in expression, there is weaker expression at invasive border which may lead to low proliferation and metastasis of the tumor and vice versa. Studies of Ozérhan et al. and Suzuki et al. show that when there is high expression at invasive border of the tumor, tumor is more likely to metastize to other organs [40,41]. Further studies with large number of samples are required to investigate the relation between homogenous expression, invasive border expression and metastasis of tumor which may be an important marker in the selection of patients diagnosed with colon carcinoma for adjuvant therapy.

CI of 40 samples was calculated and graded between 1-5 by measuring fractal dimensions of the invasive front and number of tumor cells/clusters. Results were correlated with expression of claudin proteins and clinicopathological parameters of the patients. The results do not show any significant correlation with any of the compared clinical parameters. However a trend was detected between claudin 5 expression and complexity index (p=0.06). It suggests when this protein is highly expressed, CI value of the tumor increases and high value of CI illustrate that tumor has irregular border and even separated into cells and clusters at grade 5. This result indicates that it is not only claudin 5, which is responsible for maintaining the morphology of cells and there are many other factors which are involved in establishing the integrity of tissues. Another tendency was observed between CI value and tumor wall penetration (p=0.08). Similar findings was seen in a previous study by Mannan and Hahn Stromberg, which supports the CI theory that when the tumor has an irregular border, its CI value is high, thus the penetration capability is higher in tumors with high CI value than those having low CI value and smooth borders [42]. A significant association was seen between CI and tumor wall penetration by Hahn-strömberg et al., when they observed growth pattern of the tumor and their relation to adhesion proteins and clinicopathological parameters [15]. A negatively significant association was seen between CI value and claudin 3 expression which indicates that when this protein is expressed, tumor invasive front is regular and vice versa. This finding can be used in further studies to explain this association which can be a useful prognostic marker for colon carcinoma patients.

Conclusion

In conclusion, the expression of claudin proteins (claudin 1, 3, 4, 5 and 7) was found significantly less in colon carcinoma tissue compared to the adjacent normal mucosa. We also observed that expression of claudin (claudin 1, 3, 4, 5 and 7) is not related to the growth pattern of the tumor as well as 5-years survival of the patients. However, protein expression and complexity index, both are independent prognostic markers in CRC. There is need of comparatively larger studies containing both gene and protein expression to assess the role of claudin in colon carcinoma as well as other types of carcinoma.

Authors Contribution

Abrar Ahmad carried out the image analysis, statistical analysis and drafted the manuscript together with Rahel Befekadu who also performed the immunohistochemical staining.

Shlear Askari assembled the samples, performed the sectioning for immunohistochemical staining and helped with the staining.

Victoria Hahn-Strömberg conceived of the study, design, coordination, funding and helped draft the manuscript.

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