Evaluation of Prognostic significance of TF, and β3 integrin by using Immunofluorecent staining method

Ibrahim Abdulmajeed Altamemi
Department of Microbiology, College of Medicine, Al-Qadisiya University, Iraq

*Corresponding author
Ibrahim Abdulmajeed Altamemi
Email: ibrahim.altamemi@gmail.com

Abstract: Angiogenesis, the formation of new blood vessels from pre-existing ones, is enhanced in various pathological conditions including rheumatoid arthritis, diabetic retinopathy, and cancer development. Angiogenic processes are regulated by both growth factors, and adhesion molecules, such as integrin. Current study was aimed to investigate predictive role of β3 integrin and TF protein expression in colorectal adenocarcinoma sample from Iraqi patients, through linking its expression with tumor histopathological variables (stage, grade, and lymph node involvement), by using immunofluorecent staining method. Study done on 40 colorectal cancer samples and their respective resection margins. Current study found that the expression rate of integrin β3 and TF score were significantly higher in patients than in control group, (P<0.001; P<0.001) respectively. Moreover, when CRC samples breakdown according to histopathological variables present study demonstrated that both of integrin β3 and TF count showed significant correlation with tumor stage (P<0.05 and P<0.05), grade (P<0.05, and P<0.05 ), and L.N involvement (P<0.05, and P<0.05 ) respectively. From above results one can conclude that high expression of β3 integrin and TF are associated with poor prognosis, and may play a crucial role in invasion and metastasis of colorectal carcinoma. Therefore, they may consider as a prognostic biomarkers and novel molecular therapeutic targets.

Keywords: Angiogenesis, growth factors, β3 integrin, TF protein.

INTRODUCTION:

Pathogenesis of human malignancies is tightly linked with the vascular system and at a number of ‘strategically’ important levels [1]. In this regard, two processes stand out as particularly ubiquitous and important: formation of new blood vessels (tumour angiogenesis) and activation of the coagulation system (coagulopathy), [2].

Integrin is a trans membrane glycoproteins composed of non-covalently associated α- and β-chains which recognize proteins of the extracellular matrix (ECM). The importance of ανβ3 in angiogenesis has been underscored based on the findings that ανβ3 is prominently expressed on the surfaces of endothelial cells and is highly up regulated on angiogenic blood vessels such as those in solid tumors and in granulation tissue at the base of healing wounds [1]. Recent investigations have shown that high expression of integrin β3 is positively correlated with invasion and metastasis of cancer cells and tumor angiogenesis [2, 3]. Tissue factor (TF) is an important paradigm and one of the central effectors of coagulation system and angiogenesis. TF – also known as coagulation factor III, thromboplastin, is a 47 kDa trans membrane glycoprotein [4,5]. Constitutive TF expression is restricted to sub endothelial cells that only interact with blood when vascular integrity is compromised [6]. However, it is clear that during tumourigenesis, this strict regulation of TF expression is lost [7, 8]. Different studies showed a correlation between TF and angiogenesis [9, 10]. Zhang and his colleagues found that tumor cells transfected to over express TF grew more rapidly, and established larger and more vascularized tumors than control or antisense transfectants in vivo [11]. Moreover, A significant correlation between TF expression and microvesseles density (MVD) was also found in prostate carcinomas [12], and hepatocellular carcinoma [13]. Tumors with higher TF staining showed higher MVD [13]. Thus the aim of the present study is to find if there is a prognostic significance for both of ανβ3 integrin and TF through linking its expression level with various tumor pathological variables by using immune fluorescence technique.

MATERIALS AND METHODS

Patients and Sampling:

Fourty patients with colorectal adenocarcinoma, who were confirmed...
histopathologically, were included in this study. Their age were ranged from 20-80 years. Paraffin embedded blocks of tumor and resection margins were retrieved along with the histopathological report of each patient from histopathological laboratory. For staging of the cancer, asterl-coller staging system was adopted in this study [14]. In addition, resection margins were confirmed again to be free of malignancy. Adequate thin paraffin embedded sections (5µm thick) of tumor and resection margins were prepared on positively charged slides for the immunofluorecent Technique.

Direct Immunofluorecent Detection of TF, and αvβ3 integrin.

Immunofluorescence is an antigen-antibody reaction where the antibodies are tagged (labelled) with a fluorescent dye and the antigen-antibody complex is visualized using ultra-violet (fluorescent) microscope. Fluorochromes are dyes that absorb ultra-violet rays and emit visible light. This process is called fluorescense. the fluorochromes used in this study, is fluorescein isothiocyanate (FITC), which is commonly used fluorochrome. When fluorescein (FITC) is excited by a blue (wavelength 488nm) light, it will emit a green (520nm) colour.

The steps involved are: Fixation of smear on the slide, antigen unmasking by heat epitope retrieval methods by using water bath and epitope retrieval solution with pH 9. Fallowed by treating the samples with the fallingourene labeled monoclonal antibody;

1. mouse anti-human TF (Santa cruz, USA),
2. mouse anti-human αvβ3 integrin (Santa cruz, USA).

After incubation time (1 hour), washing step was done to remove unbound excess labeled antibody and visualization under fluorescent microscope. When viewed under fluorescent microscope, the field is dark and areas with bound antibody fluoresce green. The scoring of positive cells depend on mean expression level per high powe field.

RESULTS
Tumor Sites versus their Resection Margins

According to immunofluorecent staining technique, Table 1 showed the mean score of αvβ3 integrin, mean expression level of TF in colorectal tissues of patients and control group. Mean αvβ3 integrin expression level was significantly higher in patients than in control group 74.31429±2.713466 % versus 22.55556±1.134987 %, (P<0.001), moreover mean TF count was significantly higher in patients than in control group 76.47059± 2.692649 versus 25.33333± 0.987421 (P<0.001) respectively, as shown in table [1].

<table>
<thead>
<tr>
<th>sample</th>
<th>No of samples</th>
<th>Integrin Mean ± SE</th>
<th>TF Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>40</td>
<td>74.31429±2.713466</td>
<td>76.47059± 2.692649</td>
</tr>
<tr>
<td>control</td>
<td>40</td>
<td>22.55556±1.134987</td>
<td>25.33333± 0.987421</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Correlation among Protein Expression of αvβ3 and VGF with Different Histopathological Variables

αvβ3 and TF protein expression in colorectal adenocarcinoma were also analyzed against the different pathological variables of the tumors based on Spearman’s correlation. As shown in Table 2, and 3, current study demonstrated that there were significant correlations between both αvβ3 and TF expression with tumor stage (P< 0.05, P< 0.05); and grade (P<0.05, and P< 0.05) respectively, depending on mean expression level. Moreover, a significant differences were found when we compare the expression level of both markers according to L.N involvement (P<0.05, P<0.05 respectively) table (4).

<table>
<thead>
<tr>
<th>Stage</th>
<th>No.</th>
<th>Integrin Mean ± SE</th>
<th>TF Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>51.33 ± 2.33</td>
<td>45.00 ± 3.20</td>
</tr>
<tr>
<td>B</td>
<td>11</td>
<td>67.60 ± 2.67</td>
<td>68.60 ± 2.32</td>
</tr>
<tr>
<td>C</td>
<td>14</td>
<td>85.45 ± 2.01</td>
<td>82.36 ± 1.89</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>96.50 ± 0.68</td>
<td>92.39 ± 0.59</td>
</tr>
</tbody>
</table>

* (P<0.05).
Table 3: Expression pattern of $\alpha_v\beta_3$ and TF along with tumor grade of CRC, based on spearman’s correlation (rs)

<table>
<thead>
<tr>
<th>Grade</th>
<th>No.</th>
<th>integrin Mean ± SE</th>
<th>TF Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WD</td>
<td>10</td>
<td>52.80 ± 4.57</td>
<td>53.50 ± 4.99</td>
</tr>
<tr>
<td>MD</td>
<td>18</td>
<td>74.47 ± 3.18</td>
<td>74.94 ± 3.74</td>
</tr>
<tr>
<td>PD</td>
<td>12</td>
<td>92.85 ± 2.84</td>
<td>85.60 ± 2.57</td>
</tr>
</tbody>
</table>

* (P<0.05).

Table 4: Expression pattern of $\alpha_v\beta_3$ and TF along with LN involvement of CRC, based on spearman’s correlation (rs)

<table>
<thead>
<tr>
<th>LN</th>
<th>No.</th>
<th>integrin Mean ± SE</th>
<th>TF Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free</td>
<td>14</td>
<td>64.90 ± 3.85</td>
<td>59.75 ± 3.46</td>
</tr>
<tr>
<td>Involved</td>
<td>26</td>
<td>90.11 ± 1.74</td>
<td>86.57 ± 1.60</td>
</tr>
</tbody>
</table>

* (P<0.05).

DISCUSSION

Current study had demonstrated a significant over expression of $\alpha_v\beta_3$, and TF protein expression in tumor tissue in compares with their resection margins (p<0.05, and p<0.05) respectively, table (1). This observation came in compatible with previously published data which mentioned that $\alpha_v\beta_3$ is positively correlated with angiogenic activity, invasion, and metastasis, [17] thus, it consider the leading edge of liver metastasis.

Moreover, current study showed that integrin $\beta_3$ expression rate was significantly associated with tumor stage, poor differentiation, and lymphoid node invasion, (P<0.05, P< 0.05&P<0.05) respectively; table (18-20). Thus, it came with previous studies; they mentioned that an over expression of $\beta_3$ integrin generally appears to be positively correlated with tumorigenicity. For example, expression of the $\beta_3$ integrin subunit in melanoma in situ has been found to correlate with tumor thickness, the ability to invade and metastasize, and poor prognosis [21]. Also, increased expression of integrin $\beta_3$ in gastric cancer influenced the adhesion between tumor cells and ECM. $\beta_3$ integrin may influence signal transduction, thereby changing the biological behavior of tumor cells, and enhancing the potency of infiltration and migration [22]. Previous studies have shown that integrin $\alpha_v\beta_3$ is minimally expressed on resting or normal blood vessels, but is significantly up-regulated in vascular cells within human tumors, and has been implicated in tumor-induced angiogenesis [23, 24]. Unligated integrin can act as a negative regulator of cell survival, initiating a process of “integrin mediated death” [25].

The immunoreactivity of TF in colorectal cancer with LN metastasis (Dukes’ stage C) or with liver metastasis (Dukes’ D) was significantly higher than in tumors without LN or liver metastasis (Dukes’ A, and B). These data suggest that there is a close relationship between TF and colorectal tumor metastasis. Several reports demonstrated that TF is involved in hematogenous metastasis of cancer cells [26, 27] and indirectly in tumor growth via its effect on

Fig 1: Immunofluorecent staining of TF (A), and $\beta_3$ integrin (B), in colorectal adenocarcinoma section by FITC flouro chrome (Green color) with dark background. Magnification power (40X).
angiogenesis [28]. TF from tumor cells induces fibrin formation where cancer cells are trapped to initiate a new site of tumor growth [29]. TF-mediated thrombin generation activates cell growth by acting on thrombin receptor [30]. Mastuda et al.; demonstrated direct receptor function of TF evoked by ligand (FVIIa/VII) binding [31]. Neutralization of TF function in a human melanoma cell by anti-TF antibodies inhibits tumor adherence and local growth in severe combined immuno deficient mice [31]. It is conceivable that the relationship between TF expression and tumor cell progression of CRC depends on increases angiogenesis induced by TF. Collectively, both of β3 integrin and TF expressed by cancer cells appears to act as both a regulatory target and an important mediator of oncogene driven tumor growth and neovascularization. Which might act as a target for immune modulation therapy of cancer patients?

REFERENCES

10. Staton CA, Chetwood AS, Cameron IC; Cross SS, Brown NJ, Reed MW; The angiogenic switch occurs at the adenoma stage of the adenoma carcinoma sequence in colorectal cancer. Gut 2007; 56:1426-1432.


