RESEARCH ARTICLE

Endothelial Cell Proliferation and Vascular Endothelial Growth Factor Expression in Primary Colorectal Cancer and Corresponding Liver Metastases

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Abstract

Background: Colorectal carcinoma (CRC) is one of the major causes of cancer death worldwide. Data from the literature indicate differences between the proliferation rate of endothelial cells relative to the morphology growth type, possibly due to origin of specimens (autopsy material, surgery fragments) or quantification methods. Vascular endothelial growth factor (VEGF) is a factor that stimulates the proliferation of endothelial cells. It is expressed in more than 90% of cases of metastatic CRC. Aim: The aim of this study was to evaluate the endothelial cell proliferation and VEGF expression in primary tumors and corresponding liver metastases. Materials and Methods: Our study included 24 recent biopsies of primary tumors and corresponding liver metastases of CRC cases. CD34/ Ki67 double immunostaining and RNA scope assay for VEGF were performed. Results: In the primary tumors analysis of VEGF mRNA expression indicated no significant correlation with differentiation grade, proliferative and non-proliferative vessels in the intratumoral and peritumoral areas. In contrast, in the corresponding liver metastases, VEGF mRNA expression significantly correlated with the total number of non-proliferative vessels and total number of vessels. CD34/ Ki67 double immunostaining in the cases with poorly differentiated carcinoma indicated a high number of proliferating endothelial cells in the peritumoral area and a low number in the intratumoral area for the primary tumor. Moderately differentiated carcinomas of colon showed no proliferating endothelial cells in the intratumoral area in half of the cases included in the study, for both, primary tumor and liver metastasis. In well differentiated CRCs, a high proliferation rate of endothelial cells in the intratumoral area and a lower proliferation rate in the peritumoral area were found. A low value was found in corresponding liver metastasis. Conclusions: The absence of proliferative endothelial cells in half of the cases for the primary tumors and liver metastases in moderately differentiated carcinoma suggest a vascular mimicry phenomenon. The mismatch between the total number of vessels and endothelial proliferation in primary tumors indicate that a functional vascular network is already formed or the existence of some mechanisms influenced by other angiogenic factors.

Keywords: Colon carcinoma - endothelial cell proliferation - metastasis

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Introduction

Normal tissues are characterized by the presence of blood and lymphatic vessels lined by endothelial cells exhibiting a low proliferation rate ranged between 0.1-3% of all endothelial cells which turnover daily but this percentage decline with age (Schwartz SM et al., 1973). Tumor blood vessels have an increased endothelial cells proliferation rate of 20-2000 times higher than in normal tissues (Hobson et al., 1984, Zecchin et al., 2015), representing 0.05% from the total number of human tumor proliferating cells (Kendall et al., 1999). Significant differences have been observed concerning endothelial cells proliferation rate between several tumor types, this aspect being already certified in tumors as non inflammatory breast cancer and hepatocellular carcinoma (11% and 35%, respectively) (Colpaert et al., 2003; Kendall et al., 1999; Imura et al., 2004; Quinn et al., 1993).

CRC is one of the major causes of cancer death worldwide, being the third most common diagnosed cancer in men and the second in women (Baena and Salinas, 2015). Metastatic ability of colorectal cancer cells is well certified and it is influenced by heterogeneous factors as individual’s age, dietary habits, any complaint of obesity, diabetes, previous history of cancer or intestinal polyps (Rasool et al, 2013) or by histopathologic subtypes.

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Materials and Methods

Our study included 24 recent biopsies of primary tumors and corresponding liver metastasis from patients with CRC. Biopsies were fixed in buffered formalin and embedded in paraffin. Sections from each case were stained with hematoxylin-eosin method revealed liver metastasis. Histopathological evaluation based on routine haematoxylin and eosin method revealed liver metastasis from well (8 cases), moderately (8 cases) and poorly differentiated (8 cases) CRC.

For well differentiated colon carcinoma, in primary tumor, a high proliferation rate with 3 to 15 proliferative endothelial cells were noticed. A lower proliferation rate was found in the peritumoral area. In comparison with the primary tumor, in corresponding

Table 1. Values of Proliferating Endothelial Cells According with Grading, Localization, Tumor Area

<table>
<thead>
<tr>
<th>Grading</th>
<th>Primary tumor</th>
<th>Liver metastasis</th>
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<tbody>
<tr>
<td></td>
<td>intratumoral proliferating endothelial cell</td>
<td>peritumoral proliferating endothelial cell</td>
</tr>
<tr>
<td>well differentiated</td>
<td>52</td>
<td>32</td>
</tr>
<tr>
<td>moderately differentiated</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>poorly differentiated</td>
<td>20</td>
<td>48</td>
</tr>
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</table>
liver metastasis, a low value was found. Thus, liver metastasis showed values between 1 and 3 for the proliferating endothelial cells in the intratumoral area and the absence of proliferating endothelial cells in the peritumoral area.

The moderate differentiated CRC showed the absence of proliferating endothelial cells in the intratumoral area of half of the evaluated cases, for both primary tumor and liver metastasis. A high rate of proliferating endothelial cells was found in the peritumoral area with close values for primary tumor and corresponding metastasis.

CD34/ Ki67 double immunostaining in the cases with poorly differentiated carcinoma indicated a high number of proliferating endothelial cells in the peritumoral area and a low number in the intratumoral area for the primary tumor (Figure 1 A). As a particular aspect, we noticed to these cases the presence of intussusception phenomenon associate with endothelial cell proliferation (Figure 1 B). In the corresponding liver metastasis, the proliferating endothelial cells were present in the peritumoral area and absent in the intratumoral area. The intratumoral blood vessels were small, non proliferative with collapsed lumen. The central vein adjacent to the metastasis showed a discontinuous wall and proliferating endothelial cells (Figure 1 C).

The main values of proliferating endothelial cells and the relations with grading, localization and tumor area are summarized in table 1.

In the intratumoral area of primary tumor, we found a significant correlation between the number of proliferative vessels (CD34+/Ki67+) and intratumoral proliferative endothelial cells (p=0.001). Number of non-proliferative vessels (CD34+/ Ki67-) was significantly correlated with the total number of intratumoral vessels (p=0.001) and with poor differentiated carcinoma. It was noticed that the total number of intratumoral vessels partially correlate with the differentiation degrees (p= 0.05).

Peritumoral area of primary tumor presented a correlation between non-proliferative vessels and differentiation degrees (moderate and poor differentiated type; p=0.01).

VEGFmRNA expression in primary tumor had variable scores with differentiation degree. In primary tumors, the score distribution was as follows: for well differentiated carcinoma score 3, with heterogeneous expression and distinct dots (Figure 1 D); moderately differentiated carcinoma presented a different intensity of expression in the tumor area, compact clusters and score 4 (Figure 1 E); poorly differentiated carcinoma had low intensity of reaction, visible dots and small clusters with a value of score 2 (Figure 1 F).

Analysis of VEGFmRNA expression in liver metastasis indicated a heterogeneous expression with values from 1 to 3 (Table 2). In the primary tumors no significant correlation between VEGFmRNA expression and pathological type, proliferative and non-proliferative vessels in the intratumoral and peritumoral areas was found. Opposite, in the corresponding liver metastasis, VEGFmRNA expression was significantly correlated with the total number of non-proliferative vessels (p=0.026) and total number of vessels (p=0.036).

Discussion

Tumors had preferential sites for metastasis. Thus, colon cancer has tendency to give rise to liver metastasis. At the time of the diagnosis, 25% of patients presented liver metastasis.
The addition of Bevacizumab to FOLFOX 4 (oxaliplatin, 5-fluorouracil, and leucovorin) indicated an increased of median overall survival and progression free- survival with 2.1 and 2.6 month (Giantonio BJ et al., 2007; Zhu et al., 2014). No major differences (4.7 and 4.2 month increase) were observed between administration of irinotecan, 5-fluorouracil, leucovorin and placebo treatment with foregoing treatment and bevacizumab (Dirican et al., 2014; Hurwitz et al., 2004).

The fraction of endothelial cell proliferation was described as an important factor used in the evaluation of angiogenesis (Vermeulen et al., 2002; Vermeulen et al., 1996). A study on brain metastases from NSCLC indicated a higher proliferation rate and vascular maturity comparative with primary tumors (Benjamin et al., 1999; Jubb et al., 2011). The mature blood vessels are less sensitive to Bevacizumab than immature vessels and a lower efficacy for these patients was showed.

Three distinctive morphological growth patterns were described for liver metastasis: desmoplastic, replacement and pushing (Vermeulen et al 2001). Recently, these growth patterns seems to have a prognostic significance (Nielsen et al., 2014), growth patterns having a direct correlations with recurrence free survival and other prognostic factors (Eefsen et al, 2015). A high angiogenic activity was found in the pushing growth pattern and a lower one has been noticed in a desmoplastic and replacement growth pattern. Elevated values for endothelial proliferative cells in the pushing growth pattern were found, but without a significant difference comparative to the other two types (Eefsen et al., 2012). We found, in liver metastasis values between 1 and 3 for proliferating endothelial cells for the well differentiated carcinoma, the absence of proliferating endothelial cells in half of the cases of the moderately differentiated colon carcinoma in the intratumoral area.

Our results showed a different proliferative index between the tumor blood vessel endothelium from the tumor core and its peripheral areas. This finding could partially explain the ineffective antiangiogenic and/or antiangiogenic therapy of tumor angiogenesis in liver metastasis.

Lack of CD34 immunostaining in some blood vessels lined by several Ki67-positive endothelial cells, suggests that liver metastasis tumor blood vessels are more permeable than normal blood vessels. Our study suggests that liver metastasis blood vessels are heterogeneous, do not have the same proliferative status or expression of markers concurrently and may respond in a different way to antiangiogenic therapy.

In the present study, the total number of vessels did not correlate with endothelial proliferating cells in the primary tumor. From this, it derived two hypotheses: functional intratumoral vascular network is already formed, vessels are stabilized at the moment of diagnosis or involvement of other angiogenic mechanisms dependent on other growth factors that induce the formation of new vessels. The fact that non proliferating intratumoral vessels correlates with the total number of intratumoral vessels reinforces the idea that non proliferative vessels are already functional, possibly stabilized.

Tokunaga et al. (1998) demonstrated that expression of VEGF mRNA isoforms was correlated with liver metastasis and poor prognosis in colon carcinoma. Choi et al., (2012) obtained different results: no significant relationship between the expression of VEGF, COX 2 and depth of tumor invasion, lymph node metastasis, vessel invasion, perineural invasion and liver metastasis. On the other hand, it has been showed that low VEGF165b / VEGF total ratio may be a predictive marker for bevacizumab in metastatic colorectal cancer, and individuals with high relative levels may not benefit. Initial studies of the phase III clinical trials of bevacizumab showed no predictive value for total VEGF expression or microvessel density (Bates et al., 2012; Jubb et al., 2006), suggesting that it was not the VEGF levels that determine the outcome.

In our study, no significant correlation between VEGF mRNA and pathologiical type, proliferative and nonproliferative vessels types, in the intratumoral and peritumoral areas of primary tumors was found. But, for the liver metastasis we noticed a correlation between VEGF mRNA expression and non-proliferative and total number of vessels.

In primary tumors the total number of intratumoral vessels was not correlated with endothelial cell proliferation, which can suggest that intratumoral vascular network is formed, stabilized at the moment of diagnosis. In the primary tumors no significant correlation between VEGF mRNA expression and histopathological type, proliferative and non-proliferative vessels in the intratumoral and peritumoral areas was found compared to corresponding liver metastasis in which VEGF mRNA expression was significantly correlated with total number of non-proliferative and total number of vessels.

References
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