Therapeutic approaches targeting tumor vasculature in gastrointestinal cancers

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1. ABSTRACT

Antiangiogenic therapy, especially anti-vascular endothelial growth factor (VEGF) antibody therapy, has become an important treatment option for the management of a number of human malignancies including some gastrointestinal tumors. However, there have been many cases of resistance observed against anti-VEGF antibody treatment. As to the first reason, some types of advanced colon cancers do not upregulate VEGF. As to the second reason, not a few malignancies will acquire phenotypic resistance to VEGF or its receptors after anti-VEGF antibody therapy. The molecular and cellular mechanisms associated with the resistance to VEGF-targeted agents are not fully understood. Better understanding of the mechanisms and improvement of antiangiogenic regimens to overcome drug resistance would help in the selection of those patients who are more likely to benefit from VEGF-targeted therapy. Other possible applications of anti-VEGF antibody include chemoprevention of cancer progression. It is well known that angiogenic switch and upregulation of angiogenic cascades are essential for cancer development. Therefore, prophylatic application of anti-VEGF antibody before angiogenic switch may inhibit aggressive growth of these malignancies at an initial phase.

2. INTRODUCTION

Angiogenesis is essential for tumor growth and metastasis, and depends upon the production of angiogenic factors by tumor cells, stromal cells, infiltrating immune cells and vascular components (1, 2). Vessel counts have been shown to correlate with an increased incidence of metastases in many tumors. Of the known angiogenic factors, vascular endothelial growth factor (VEGF) has been reported to play an important role in regulating angiogenesis of many cancers. Since we first reported that vessel counts, expression of VEGF and VEGF receptor (VEGFR), and kinase insert domain protein receptor correlated with metastasis and proliferation of human colon cancer (3), many researchers have confirmed that VEGF is an important angiogenic factor in the patients with colon cancer, supporting the modality of VEGF-targeted therapy for this disease. Cumulative investigations have shown that anti-VEGF antibody and VEGFR antagonist inhibit tumor growth and metastasis in animal models (4, 5). Based on the favorable results, many clinical trials of these VEGF-targeted drugs have been done in several types of malignancies such as breast, lung and colon cancers. Among them, phase II and III studies of the recombinant human monoclonal antibody against VEGF (bevacizumab)

Table 1. Examples of clinical trials of bevacizumab in eshophageal and gastric cancer

Docetaxel, Cisplatin, Irinotecan and Bevacizumab (TPCA) in Metastatic Esophageal and Gastric Cancer (Dana-Farber Cancer Institute)
Cisplatin, Irinotecan and Bevacizumab (PCA) Versus Docetaxel, Cisplatin, Irinotecan and Bevacizumab (TPCA) in Metastatic Esophageal and Gastric Cancer (Dana-Farber Cancer Institute)
Phase I/II Study of Weekly Docetaxel and Cisplatin Together With Capecitabine and Bevacizumab in Advanced Gastric Cancer (Rabin Medical Center)
Oxaliplatin/Irinotecan/Bevacizumab Followed by Docetaxel/Bevacizumab in Inoperable Locally Advanced or Metastatic Gastric Cancer Patients (Pfizer, Sanofi-Aventis and Hoffman-La Roche)
A Study of Avastin (Bevacizumab) in Combination With Xeloda (Capecitabine) and Cisplatin as First-Line Therapy for Advanced Gastric Cancer (Hoffman-La Roche)

"The detailed information is available at http://clinicaltrials.gov"

Table 2. Examples of clinical trials of bevacizumab in pancreatic cancer.

Gemcitabine With or Without Bevacizumab in Treating Patients With Locally Advanced or Metastatic Pancreatic Cancer (National Cancer Institute)
Gemcitabine and Bevacizumab in Treating Patients With Pancreatic Cancer That Has Been Completely Removed By Surgery (National Cancer Institute)
Bevacizumab, Erlotinib and Capecitabine for Advanced Pancreatic Cancer (M.D. Anderson Cancer Center)
Combining Erlotinib Plus Bevacizumab and Gemcitabine Plus Capecitabine to Treat Advanced Pancreatic Cancer (Hoffman-La Roche)
Gemcitabine, Infusional 5 Fluorouracil and Bevacizumab in Patients With Advanced Pancreas Cancer (Ohio State University Comprehensive Cancer Center)

"The detailed information is available at http://clinicaltrials.gov"

showed favorable outcomes (6, 7). In the phase III study, median survival time and duration of remission in the irinotecan (CPT-11) + 5-fluorouracil (5-FU) + leucovorin (LV) with bevacizumab group was much longer than those in the CPT-11 + 5FU + LV alone group of patients with metastatic colon cancer. Currently, bevacizumab is widely accepted as a key chemotherapeutic drug for many solid tumors including lung, kidney, and breast cancers as well as colon cancer. However, therapeutic effects of VEGF-targeted molecules seem to be limited and most of the patients die from the diseases. In this review, we discuss the problems of anti-VEGF antibody therapy and new approaches of antiangiogenic therapy.

3. DISCUSSION

3.1. Clinical trials of bevacizumab in gastrointestinal cancers

3.1.1. Esophageal and gastric cancers

There are 21 on-going clinical studies. The detailed information is available at http://clinicaltrials.gov (Table 1). Most of them are phase II studies of combination therapies using chemotherapeutic drugs such as docetaxel, paclitaxel, cisplatin and capecitabine in patients with advanced and/or metastatic esophageal and gastric cancers.

Gastric cancer prognosis is dependent at least in part on pathological types and disease stages. For example, intestinal type of gastric cancer tends to be exophytic, and frequently involves the liver by hematogenous metastasis (8, 9). In contrast, diffuse type of gastric cancer is more invasive, and tends to spread in the peritoneal cavity. The key factors responsible for liver metastasis and peritoneal dissemination have not been fully identified, yet. We previously reported that the processes of growth and metastasis in intestinal type of gastric cancers were more angiogenesis-dependent than in diffuse type cancers (10). The significant correlation between VEGF expression and vessel counts implied that VEGF-induced angiogenic response plays a critical role in the progression of intestinal type of gastric cancers. It seems to be critical to understand which molecules play a principal role in angiogenesis in different pathological types of tumors. We investigated

the biological behavior of a special type of tumor, i.e., alpha-fetoprotein (AFP)-producing type of gastric cancer (AFP-GC). AFP-GC is known to be highly angiogenic, frequently metastasize to the liver and lead to poor prognosis. We compared vessel counts and expression levels of VEGF and AFP between AFP-GC and non-AFP-GC tissues, and found that AFP expression was significantly correlated with VEGF expression and vessel counts in AFP-GC group (11). Furthermore, we investigated angiogenic activity of AFP-GC in xenotransplantation model. Successful suppression of angiogenesis by anti-AFP antibody in our model suggested that AFP itself might upregulate angiogenic activities of AFP-GC in a direct manner, and that AFP antibody might exert antiangiogenic effects, inhibiting hematogenous metastasis to the liver (11). From these results, we suggest that we may select patients with gastric cancers such as intestinal type and AFP-GC for this treatment protocol.

3.1.2. Pancreatic cancer

Since phase III trial of bevacizumab in combination with gemcitabine in patients with metastatic pancreatic cancer did not show significant improvement in overall survival (12), most clinical studies using gemcitabine have been terminated. Currently, more than 10 clinical studies of combinations of bevacizumab and other chemotherapeutic drugs such as capecitabine (pro-drug of 5-FU) and other molecular targeting drugs such as erlotinib (epidermal growth factor receptor [EGFR]-inhibitor) are going on (Table 2).

Phase II study of bevacizumab and gemcitabine in 52 patients with advanced pancreatic cancer showed that 11 patients (21%) had confirmed partial responses, and 24 (46%) had stable disease (13). The 6-month survival rate was 77%. Median survival was 8.8 months; median progression-free survival was 5.4 months. Pretreatment plasma VEGF levels did not correlate with outcomes. Grade 3 and 4 toxicities included hypertension in 19% of the patients, thrombosis in 13%, visceral perforation in 8%, and bleeding in 2%. However, phase III study of those drugs has so far failed to demonstrate a survival benefit. Some researchers have pointed out the heterogeneity of pancreatic cancer (14, 15).

Table 3. Examples of clinical trials of bevacizumab in colon cancer.

Bevacizumab and Cetuximab in Combination With FOLFOX6 in Patients With Metastatic Colorectal Cancer(Sarah Cannon Research Institute)
Fluorouracil, Leucovorin, and Oxaliplatin With or Without Bevacizumab in Treating Patients Who Have Undergone Surgery for Stage II or Stage III Colon Cancer (National Cancer Institute)
Phase II Study of Bevacizumab, Capecitabine and Oxaliplatin in Colon Cancer (Asan Medical Center)
Second-Line Combination Chemotherapy With or Without Bevacizumab in Treating Patients With Metastatic Colorectal Cancer Who Have Received First-Line Chemotherapy and Bevacizumab (National Cancer Institute)
Erlotinib, Bevacizumab, and Combination Chemotherapy in Treating Patients With Metastatic or Locally Advanced Colorectal Cancer (National Cancer Institute)

3.1.3. Colon cancer

More than 200 clinical studies are recruiting patients (Table 3). Most of them are combination studies of bevacizumab with chemotherapeutic drugs such as capecitabine, irinotecan and/or other molecular targeting drugs such as erlotinib and another EGFR-inhibitor cetuximab. These clinical studies include second lines of combinations with bevacizumab and chemotherapeutic drugs, and adjuvant settings in patients who have undergone surgery.

Since Hurwitz *et al.* reported that the combination therapy of bevacizumab with CPT-11, 5-FU and leucobolin showed excellent survival gain in patients with colon cancer (7), bevacizumab has been regarded as a key drug for locally advanced and metastatic colon cancers. Based on the favorable effect of bevacizumab in advanced cases, further treatment modalities of VEGF-targeting drugs in colon cancer are considered as adjuvant therapy and chemoprevention. We previously reported that the recurrent rates were more frequent in the patients with high VEGF expression than in those with low VEGF expression among lymph node-negative colorectal cancers, and that mRNA upregulation of metastasis-related genes such as angiogenic factors, metalloproteinases, growth factors and adhesion molecules occurred just prior to liver metastasis in an orthotopic mouse model of human colon cancer (16, 17). These clinical and experimental data strongly suggest that chemoprevention of recurrence by bevacizumab in the combination with chemotherapeutic drugs will improve the outcomes of early stage patients with high VEGF colorectal cancers.

3.2. Resistance to anti-VEGF antibody
3.2.1. Mechanism

There are many cases of resistance to bevacizumab before and after antiangiogenic therapies in advanced gastrointestinal tumors. Although the mechanism of drug resistance may be complicated, it depends in part on pathological nature of tumor cells in each case. Some advanced gastrointestinal tumors do not upregulate VEGF (intrinsic resistance). Other mechanisms include altered sensitivity. Tumors that are originally sensitive to anti-VEGF or anti-VEGFR therapy may acquire phenotypic resistance after bevacizumab therapy (acquired resistance).

With regard to intrinsic resistance, Shojaei *et al.* demonstrated that the bone marrow-derived CD11b$^+$ Gr1$^+$ myeloid cells played an important role in the tumors that were inherently refractory to anti-VEGF therapy. In the experiments, they used transplanted mouse models of tumor cell lines EL4 (lymphoma) and LLC (lung carcinoma) that were resistant to anti-VEGF antibodies. The numbers of bone marrow-derived CD11b$^+$ Gr1$^+$ cells in EL4 and LLC tumors were significantly higher than those

in B16F1 tumors (melanoma) that were sensitive anti-VEGF therapy. Sensitivity of B16F1 tumors to anti-VEGF therapy was not significantly affected by admixing B16F1 cells with CD11b$^+$ Gr1$^+$ cells primed by B16F1 tumors. However, when B16F1 cells were mixed with the CD11b$^+$ Gr1$^+$ cells primed by EL4 or LLC cells, the transplanted B16F1 tumor showed refractoriness to anti-VEGF treatment (18).

As to the acquired resistance, Casanovas *et al.* showed that the upregulation of the angiogenesis stimulator basic fibroblast growth factor (bFGF) within the tumor after treatment with anti–VEGFR2 antibody therapy. This effect is probably caused by the elevated levels of hypoxia induced by the antibody treatment (19).

Apart from the studies in rodent models, clinical investigations of resistance to anti-VEGF therapy have also been reported. Batchelor *et al.* investigated the mechanism of refractoriness to AZD2171, an oral tyrosine kinase inhibitor of VEGFRs, in glioblastoma patients. Increases in the circulating levels of bFGF and CXCL12 (also named SDF-1α) were observed when tumors progressed on VEGF-targeted therapy (20). The study suggests that other factors than VEGF such as bFGF and CXCL12 may play an important role in stimulating alternative angiogenic cascades of glioblastoma vessels in the patients treated with anti-VEGF agents.

Delta-Notch pathway is another important regulator of embryonic angiogenesis. Delta-like 4 (Dll4) is an endothelial cell-specific Notch ligand. Haploinsufficiency of Dll4 in mice results in embryonic lethality due to defective vascular development, suggesting that Dll4 is indispensable for vascular development and remodeling. Dll4 is thought to act downstream of VEGF. Therefore, Dll4/Notch blockade might be beneficial in tumors that either are intrinsically resistant or acquire resistant to anti-VEGF therapy (21). To support the notion, inhibition of Dll4 results in tumor suppression. Remarkably, Dll4 inhibitor-treated tumors show non-productive angiogenesis with hyper-proliferative endothelial cells and absence of pericyte. Several lines of investigations demonstrated that an inhibitor of Dll4-Notch system was effective in the tumors that were resistant to VEGF inhibitors. Preclinical studies showed that the blockade of Dll4 was effective in inhibiting tumors that were resistant to anti-VEGF therapy.

3.2.2. How to conquer the resistance

The inhibition of VEGF signaling can lead to a compensatory increase in expression of other angiogenic

Table 4. Effects of treatment with KKLS (VEGF dependent)(37)

Treatment	Size of primary tumor	Liver metastasis rate	Vessel count
Anti-VEGF antibody	12.5+_4.5 mm*	1/10*	12.5 +_ 4.8*
DFMO	13.8 +_ 5.9 mm	3/10*	17.4 +_ 5.1*
Combination	9.5 +_3.4 mm**	0/10	8.1 +_ 2.8**
Control	17.8 +_ 6.3 mm	7/10	27.5 +_8.1

* $p<0.05$ ** $p<0.001$ (compared to control group)

Table 5. Effects of treatments with KM12SM (VEGF-independent) (37)

Treatment	Size of primary tumor	Liver metastasis rate	Vessel count
Anti-VEGF antibody	15.3 +_ 5.7 mm	5/10	20.3 +_ 5.7
DFMO	13.3 +_ 5.7 mm	3/10*	16.1 +_ 5.1*
Combination	12.9 +_ 4.5 mm*	2/10*	13.8 +_ 3.8**
Control	18.3 +_ 6.1 mm	7/10	26.2 +_ 7.3

* $p<0.05$ ** $p<0.001$ (compared to control group)

factors. As we mentioned above, bFGF and CXCL12 are reported to play a role in VEGF-independent angiogenesis in some tumor types. In addition, placental growth factor (PlGF) has been shown to be increased in plasma following blockade of VEGF signaling (20, 22). It has been reported that PlGF neutralization by monoclonal antibody is effective for both VEGF-sensitive and VEGF-resistant tumors (23). It is critically important to understand which angiogenic factors play a pivotal role in the tumor of each patient at different stages, and it is not enough for antiangiogenesis therapy to target VEGF alone.

3.2.2.1. Metronomic chemotherapy

The addition of non-VEGF-targeted antiangiogenic drugs has been proposed to resolve this problem. Folkman and Kerbel groups have reported that frequent low dose chemotherapy induces antiangiogenesis by apoptosis of endothelial cells in their experimental models, calling this approach 'metronomic chemotherapy' (24, 25). It has been reported that the combination of low-dose chemotherapy and bevacizumab delayed breast cancer progression by an average of five and a half months, compared to two months with the low-dose chemotherapy alone. This combination therapy was quite well tolerated in a pilot study of 55 breast cancer patients (26).

Currently, metronomic dosing principles are not defined and the dose range is empirically evaluated. A reliable dose-finding system for metronomic chemotherapy should be further studied. Shaked *et al* reported a new dose-finding system by monitoring viable circulating VEGFR2$^+$ endothelial precursor cells (CEPs) in peripheral blood (27), which would be a clinical marker for the evaluation of antiangiogenic drug activity (28). They also concluded that the optimal dose for metronomic chemotherapy was closely correlated with the maximum reduction in CEPs. Lam *et al* reported that human umbilical vein endothelial cells (HUVEC) were more sensitive than some cancer cell lines to low dose chemotherapeutic drugs such as temozolomide, estramustine and paclitaxel (29). The optimal dose for metronomic chemotherapy selectively damaged HUVEC, suggesting that this approach in the combination with antiangiogenic molecules would increase efficacy, reduce toxicity, and yield better outcomes.

3.2.2.2. Non-VEGF-targeted antiangiogenic drug

In the other approaches besides metronomic chemotherapy, therapeutic efficacy of combination therapy, i.e., the partnership of VEGF-targeted agent and non-VEGF-targeted antiangiogenic drugs, have also been reported.

We selected α-difluoromethylornithine (DFMO) as a non-VEGF-targeted antiangiogenic drug (30, 31). Polyamines, primarily putrescine, spermidine and spermine are known to play critical roles in cell proliferation including tumor growth, differentiation and maintenance as well as neoplastic transformation including carcinogenesis of mammalian cells. DFMO is a specific and irreversible inhibitor of ornithine decarboxylase (ODC), a key enzyme converting ornithine to putrescine in polyamine biosynthesis. Biosynthesis of polyamines is closely linked to the cellular synthesis of DNA, RNA and protein (32). Rapidly growing cells such as tumor cells and regenerating cells produce greater amounts of polyamines than normal tissues. Therefore, DFMO has been used in clinical trials for chemoprevention of several tumors such as colon cancer (33, 34), colon polyp (35), and cervical intraepithelial neoplasia (36). We hypothesized that chemopreventive activities of DFMO included antiangiogenesis, and showed that DFMO inhibited the growth of HUVEC *in vitro,* and that DFMO also reduced vessel counts followed by the inhibition of growth and liver metastasis of transplanted gastric cancer KKLS in nude mice *in vivo* (30). In another experiment, we demonstrated that anti-VEGF antibody selectively inhibited angiogenesis, tumor growth and liver metastasis of VEGF-dependent KKLS model (37). As expected, the combination of anti-VEGF antibody with DFMO exerted synergistic effects on this VEGF-dependent tumor model (37). On the other hand, anti-VEGF antibody alone failed to inhibit angiogenesis in a VEGF-independent tumor (KM12SM) which was originated from human colon cancer. However, anti-VEGF antibody in combination with DFMO significantly inhibited angiogenesis, tumor growth and liver metastasis, and induced tumor apoptosis (Tables 4 and 5) (37). These results suggest that this combination of VEGF-targeted and -non-targeted antiangiogenic drugs may solve the problems associated with intrinsic resistance, and may well be applicable for both VEGF-dependent and -independent tumors. It is a subject for future study whether this combination therapy also exerts antiangiogenic effects in the tumors with acquired resistance to anti-VEGF therapy due to prolonged administration of bevacizumab.

Table 6. Vessel density, intensity of VEGF mRNA, size of stomach tumor and liver metastasis among the pre-AS treatment, post-AS treatment and control groups (45).

	Vessel density (mean ±SD)	Intensity of VEGF mRNA (mean ±SD)	Size of stomach tumor (mean (mm) ±SD)	Liver metastasis
Pre-AS treatment group	23.8 +_5.1*	23.5 +_9.5*	12.1 +_5.1	0/10**
Post-AS treatment group	33.5 +_5.5	65.3 +_13.5	16.2 +_5.7	4/10
Control group	41.2 +_13.5	78.3 +_17.3	18.3 +_7.3	5/10

* p<0.001, ** p<0.01 (compared to the control group)

3.3. Angiogenic switch in gastrointestinal cancers
3.3.1. Colorectal cancer

It is essential for tumors to develop the angiogenic phenotype in order to grow and metastasize. The conversion of a small, poorly-vascularized tumor to a growing well-vascularized tumor has been termed "angiogenic switch". It is well known that the angiogenic switch, i.e., upregulation of angiogenesis, is essential for cancer development. Hanahan and Folkman have reported that angiogenic switch occurs at early stages of tumor development in transgenic mouse models and at premalignant stages of human malignancies including breast cancer and cervical squamous cell cancer (38). Among various human solid tumors, the progression pattern of colon cancer is a good model for investigating the angiogenic switch. Although certain exceptions should be noted, there are well defined progression steps in the adenoma-carcinoma sequence: I) adenoma with severe dysplasia and villous architecture associated with genetic instability may develop carcinoma, II) Carcinoma in situ (Tis) may often arise in adenoma, and the spread pattern is superficial without vascular or lymphatic invasion, III) Carcinoma invading submucosa (T1) has the risks of hematogenous and lymphatic metastasis, IV) Advanced cancer (T2) invading muscularis propria or subserosa increases the risks of metastasis and recurrence. Several researchers have studied angiogenesis and angiogenic factors in human colon cancer with sometimes different results. Wong et al. reported that activation of VEGF as the molecular basis for the induction of angiogenesis occurred in the pre-malignant phase of colorectal tumor development (39). Kakolyris et al. also reported that vessel counts in cancers were significantly higher than those in adenomas (40). Kondo et al. reported that vessel density and VEGF expression were upregulated in association with tumor progression from adenoma to noninvasive colorectal cancer (41).

We and another group investigated various factors previously shown to be important in colon cancer angiogenesis (42-44) to clarify whether the upregulation of these key factors occur simultaneously at a specific stage in tumor progression (44). Our immunohistochemical analysis showed that vessel counts and the staining intensities of VEGF, platelet-derived endothelial cell growth factor PD-ECGF and matrix metalloproteinase (MMP)-7 were significantly different between Tis and T1, suggesting that these factors upregulate simultaneously at the time of conversion from non-invasive to invasive phenotype in colon cancers (Figure 1)(44). On the other hand, there were no differences in the expression intensities of these factors at any other stages in colon cancers with the exception of PD-ECGF in which stronger expression intensity was observed in T2 compared to T1.

We also showed that mRNA upregulation of metastasis-related genes occurred just prior to liver metastasis in an orthotopic mouse model of colon cancer (17). In this experiment, human colon cancer KM12SM cells were implanted into cecal wall of nude mice (orthotopic model). During the first two weeks after implantation, KM12SM tumor grew progressively in the mucosa and submucosal layers. By the third week, they invaded the muscularis propria and then the serosa. All mice undergoing cecectomy at 1-2 weeks were cured, whereas those undergoing cecectomy at later weeks were dead. By in situ hybridization, the expression levels of bFGF, EGFR and type IV collagenases were low in the early cecal tumors but increased just before the tumor invaded the muscularis propria. These data suggest that the upregulation of angiogenic factors, i.e. induction of angiogenic switch, precedes tumor cell invasion, and that this conversion accelerates tumor metastasis.

We further studied whether anti-VEGF antibody inhibits angiogenic switch and liver metastasis of orthotopic xenograft model with site-dependent tumor of VEGF. Anti-VEGF antibody treatment before angiogenic switch could efficiently inhibited tumor vasculature and liver metastasis in this model, whereas the treatment after angiogenic switch could not suppress liver metastasis in about half of the cases (Table 6)(45). From these results, we propose that anti-VEGF antibody may have another therapeutic option as a chemoprevention drug in patients at a high risk of developing colon cancer, especially those with premalignant lesions such as familial polyposis and long-term ulcerative colitis.

3.3.2. Other cancers

Kitadai et al. reported that angiogenic switch is a very early event in the development of invasive esophageal cancer, and that several angiogenic factors regulate angiogenesis in a time-dependent manner during different stages of carcinogenesis (46). They found that VEGF and PD-ECGF were upregulated in pre-malignant dysplastic lesion with initial increase of vessel count, and that bFGF and interleukin (IL)-8 were elevated successively in later stages of esophageal cancer in accordance with significant increase of vessel count. The notion of angiogenic switch in pre-malignant conditions of esophageal cancer has been supported by some other groups (47, 48).

Several investigators have reported that the angiogenic switch in pancreatic malignancies is also an early event in both a murine insulinoma model and human pancreatic cancers (49-51). Nozawa et al. demonstrated that MMP-9^{+} neutrophils were predominantly found inside angiogenic islet dysplasias and tumors in RIP1-Tag2 mice, and that these infiltrating neutrophils played a crucial role
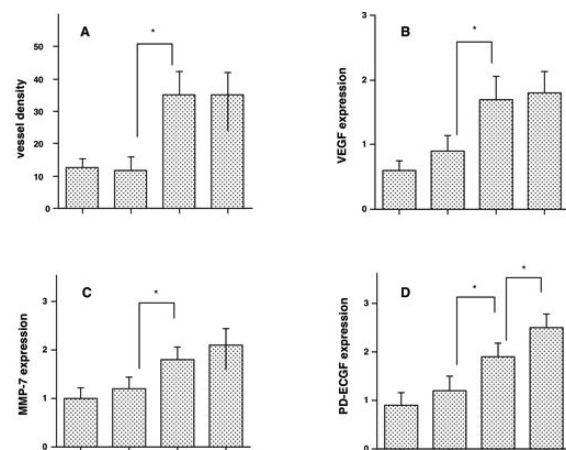
Figure 1. Vessel density (A) and the expression intensity of VEGF (B), MMP-7 (C), and PD-ECGF (D) in adenoma, Tis, T1 and T2. There are significant increases in all factors studied between Tis and T1 cancers and increases in PD-ECGF expression between T2 and T1 cancers (44). p<0.01

in activating angiogenesis during the early stages of carcinogenesis in this model (49). In the study on angiogenic switch in human pancreatic cancer, Abdollahi *et al.* developed transcriptome-based angiogenic signaling network in endothelial cells that were downregulated after endostatin and upregulated after VEGF/bFGF treatment. They identified several key molecules such as peroxisome proliferative activated receptor δ (PPARδ) and MMP-1. Comparison among human normal pancreas, pancreatitis and pancreatic cancer tissues, PPARδ was elucidated as an important proangiogenic "hub node". These selected molecules are expected to be involved in angiogenic switch of several human malignancies besides pancreatic cancer, and further studies on the signaling network are necessary to identify new therapeutic targets and improve therapeutic strategies, especially for those who obtain resistance to previous antiangiogenic therapies.

4. PERSPCETIVE

Anti-VEGF antibody therapy is currently available as the most popular anti-angiogenic therapeutic modality in several types of human malignancies. As we discuss in this review, anti-VEGF therapy is not only a treatment option for advanced tumors but may also potentially work for chemoprevention for gastrointestinal cancers. Tumor stages, immune responses, genetic backgrounds, and other clinicopathological factors, in addition to the nature of tumor cells contribute to tumor-specific angiogenic microenvironment and intrinsic/acquired resistance. Further studies are necessary to find an optimal combination of VEGF-targeted and non-VEGF-targeted drugs in the treatment of gastrointestinal cancers and other malignancies.

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