Targets in the microenvironment of rectal cancer

Tamas, Karin Rita

7

Summary

Summary

Drug development in oncology was initially focused on inducing DNA damage in proliferating cancer cells, with currently more emphasis on targeted agents and immunotherapy. Tumor cells and their microenvironment interact and this gives opportunities for potential targets for treatment. New approaches are critical as many patients still have recurrence disease and develop resistance to their cancer treatment.

Angiogenic factors and chemokines

The aim of this thesis was to explore targets in the microenvironment of rectal cancer with a focus on angiogenic growth factors and chemokines.

In this thesis, two key pathways of tumor-microenvironment interaction were studied with emphasis on rectal cancer, namely the vascular endothelial growth factor A (VEGFA)-mediated pathway promoting tumor angiogenesis, and the chemokine ligand 12 (CXCL12)/chemokine receptor 4 (CXCR4) signaling pathway primarily directing cellular migration toward distant metastatic sites.

Chapter 1 gives the outline of the content of the thesis.

The clinical and biological differences and similarities between rectal and colon cancer are presented in a literature review in **Chapter 2**. Several hallmarks establish rectal cancer as distinct from colon cancer. The rectum and colon have a different embryological origin and anatomy, and have a different function. Moreover, rectal and colon cancer differ in their metastatic pattern, and contain a different set of drug targets, such as v-raf murine sarcoma viral oncogene B (BRAF), which is preferentially mutated in proximal colon carcinomas, and the epidermal growth factor receptor (EGFR), that is prevalently amplified or overexpressed in the distal colorectum. Differences in anatomy led to different surgical approaches and administration of neoadjuvant (chemo)radiotherapy solely for rectal cancer.

Adjuvant systemic treatment increases overall survival of patients with stage III colon cancer, whereas its role in rectal cancer is not proven. Metastatic rectal and colon cancer are commonly regarded as one entity, and treated alike. Insights in difference between rectal and colon cancer are of importance, since they may provide guidance for the design of novel clinical approaches.

In **Chapter 3** we evaluated the efficacy and tolerability of preoperative radiotherapy, followed by systemic capecitabine-oxaliplatin (CapeOx) treatment in combination with bevacizumab – a humanized monoclonal antibody against VEGFA – and subsequent radical surgical treatment of all tumor sites of in 50 de novo metastatic rectal cancer patients. Treatment comprised radiotherapy (5x5 Gy), followed within 2 weeks by bevacizumab (7.5 mg/kg, day 1) and oxaliplatin (130 mg/m^2, day 1) intravenously and capecitabine (1000 mg/m^2 twice daily orally, days 1-14) for up to six 3-weekly cycles. Surgery and/or radiofrequency ablation (RFA) was performed 6 to 8 weeks after the last bevacizumab dose. The most commonly observed clinical stage was cT3N1-2 in 64% of the patients, with liver metastases. Of 50 included patients, 42 had liver metastases, five had lung metastases, and three had both liver and lung metastases. Preoperative radiotherapy was given to all 50 patients, and subsequent preoperative bevacizumab-CapeOx treatment was started in 49 (98%) patients. Forty two (84%) patients received all six cycles of bevacizumab-CapeOx. Toxic effects were tolerable. The most common nonsurgical grade ≥3 toxic effects were diarrhea and pulmonary embolism. The most frequent grade 1-2 adverse reactions to the systemic drug treatment were fatigue, sensory neuropathy and nausea. No metastatic disease progression was detected by radiological reassessment during or at the completion of preoperative treatment. Radical surgical treatment of all tumor sites (R0) was possible in 36 of the 50 patients. The most frequent postoperative complications within 60 days after surgery were wound and abdominal cavity infections. No treatment-related deaths occurred. A

complete pathologic response of the primary tumor was found in 26% of patients, and in 16% near-complete response, with only a few residual tumor cells present. Rectal tumor downstaging occurred in 47% of patients who had primary tumor resection. The 2-year overall survival rate was 80% in the intent-to-treat group (n=50; 95% confidence interval (CI), 66.3 to 90.0%). The 2-year recurrence rate was 64% (23/36 patients; 95% CI, 49.8 to 84.5%) after R0 resection. Median time to recurrence was 13 (range 7-20) months. Local pelvic relapse occurred in two out of the 23 patients, and distant recurrences – mainly in the liver/lungs – in 21 out of the 23 patients. This study shows that radical surgical treatment of all tumor sites carried out after short-course radiotherapy and systemic bevacizumab-CapeOx combination treatment is a feasible and potentially curative approach in primary metastasized rectal cancer patients. This approach may enable both treatment of metastatic disease and good control of the primary rectal tumor.

Results in Chapter 3 show that about 65% of the primary metastasized rectal cancer patients experienced, mainly distant, disease recurrence within 2 years after radical surgical treatment of all tumor sites. Therefore, in **Chapter 4** we studied the expression of CXCR4 and CXCL12 in 46 primary rectal tumors before and after patients underwent local radiotherapy and systemic treatment with bevacizuma-CapeOx and subsequent radical surgery. At diagnosis, cytoplasmic CXCR4 and CXCL12 expression in cancer cells was present in 89% and in 81% of the tumor samples, respectively. Nuclear CXCR4 and CXCL12 expression in cancer cells was observed in 30% and 35% of the cases. Stromal cells expressed cytoplasmic CXCR4 and CXCL12 in 98% and 86% of the tumor samples respectively. Nuclear CXCR4 and CXCL12 expression was present in stromal cells in 14% and 16% of the tumor samples. At baseline, there were no differences in CXCR4 or CXCL12 expression between the nine patients that had and the 30 that did not have a pathologic complete response to treatment. After treatment, nuclear CXCL12 expression in cancer cells was present in 79%

of residual tumors, as compared to only 31% of the paired tumor samples expressing nuclear CXCL12 before treatment (*P*=0.001). In conclusion, CXCR4 and CXCL12 are extensively expressed in rectal tumors of patients presenting with metastatic rectal cancer, and treatment further upregulates expression of CXCL12. These data indicate that the CXCR4 receptor and its ligand CXCL12 are potential therapeutic targets in rectal cancer.

Expression of placental growth factor (PlGF) – a VEGFA homolog – is related preclinically to tumor angiogenesis and survival of cancer cells, and correlates with poor survival in colorectal cancer patients (1-3). Moreover, bevacizumab alone or in combination with radiotherapy or chemotherapy increases circulating PlGF levels in (colo)rectal patients (4, 5). Hence, in **Chapter 5** we studied the VEGFA and PlGF protein signature, and the mean vessel density (MVD) in rectal tumors of 46 patients before and after they underwent local radiotherapy and systemic bevacizumab-Capox and participated in the study described in Chapter 3. At diagnosis, VEGFA was expressed in 91% of the tumor samples in the cytoplasm and in 50% in the nucleus of tumor cells, whereas PlGF was expressed in 74% only in the cytoplasm of tumor cells. Simultaneous VEGFA and PlGF expression was present in the cytoplasm of tumor cells in 65% of the tumor samples. PlGF expression was absent in tumor-adjacent stromal cells in 96% of the tumor samples. There were no differences in VEGFA expression and MVD at baseline between the nine patients that had and the 30 who did not have a pathologic complete response. All nine patients with pathologic complete response had PlGF expression in tumor cells at baseline, as did 19 of the 30 patients with residual tumor. In the 11 rectal tumors of patients without PlGF expression at baseline no pathologic complete response was found. After treatment, nuclear VEGFA expression in tumor cells and MVD were lower in the residual tumors as compared to pretreatment values (15% vs. 56%, *P*=0.024, and 10.3 (±4.2) vs.16.4 (±6.0), *P*<0.0001). PlGF expression in the residual tumors did not differ from the pretreatment values. This study shows that rectal

tumors of newly diagnosed stage IV patients express not only the well-known VEGFA but also PlGF extensively. In view of the proangiogenic function of PlGF and in conjunction with the clinical efficacy and safety profile of available PlGF inhibitors, a possible implication of this finding is that PlGF blockade might be of interest to study in rectal cancer patients.

PlGF inhibition showed antitumor activity in preclinical tumor models resistant to VEGF receptor (VEGFR) inhibitors (6). These findings led to the clinical development of RO5323441, a humanized monoclonal antibody against PlGF. Since its administration in humans did not lead to dose-limiting toxicities, no optimal therapeutic dose was defined (7, 8). In vivo RO5323441 imaging could allow dynamic non-invasive assessment of target (PlGF) saturation during treatment, thus support rational drug development. Therefore, in **Chapter 6** we labeled RO5323441 with ^{89}Zr to develop a PlGF-specific PET tracer and investigate ^{89}Zr-RO5323441 tumor uptake and organ distribution in human tumor bearing mice. We used three different tracer doses, as well as three different imaging and biodistribution time points, two tumor models with different PlGF expression levels, an unspecific IgG control, and a RO5323441 pretreatment dose. The fluorescent Cy5-RO5323441 was injected to study the intra-tumor distribution of RO5323441 with fluorescence microscopy. RO5323441 could be readily labeled with ^{89}Zr with high specific activity (up to 1 GBq ^{89}Zr/mg RO5323441), a radiochemical purity of >95%, stability in human serum at 37 °C of >1 week, and a fully preserved immunoreactivity. ^{89}Zr-RO5323441 showed a time- and dose-dependent tumor accumulation. We found that uptake in PlGF-expressing Huh7 xenografts at 10 μg ^{89}Zr-RO5323441 was 8.2 ± 1.7 % injected dose (ID)/cm^3 at 144 hours after injection, whereas in PlGF not expressing ACHN xenografts it was 5.5 ± 0.3 %ID/cm^3 (P=0.03). RO5323441 pretreatment (20 mg/kg) of Huh7 xenograft-bearing mice reduced ^{89}Zr-RO5323441 tumor uptake to the level of nonspecific ^{111}In-IgG uptake. Cy5-RO5323441 was present in the tumors mainly in the microenvironment. Overall,

these data showed a PlGF specific, time- and dose-dependent RO5323441 uptake in tumors. This supports the feasibility of ^{89}Zr-RO5323441 PET scanning for clinical studies of the PlGF antibody RO5323441.

References

(1) Dewerchin M, Carmeliet P. PlGF: a multitasking cytokine with disease-restricted activity. Cold Spring Harb Pespect Med 2012;2:a011056.

(2) Snuderl M, Batista A, Kirkpatrick ND, et al. Targeting placental growth factor/neuropilin 1 pathway inhibits growth and spread of medulloblastoma. Cell 2013;152:1065-1076.

(3) Escudero-Esparza A, Martin TA, Davies ML, Jiang WG. PGF isoforms, PlGF-1 and PGF-2, in colorectal cancer and the prognostic significance. Cancer Genomics Proteomics 2009;6:239-246.

(4) Willett CG, Duda DG, Di Tomaso E, et al. Efficacy, safety, and biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: A multidisciplinary phase II study. J Clin Oncol 2009;27:3020-3026.

(5) Kopetz S, Hoff PM, Morris JS, et al. Phase II trial of infusional fluorouracil, irinotecan, and bevacizumab for metastatic colorectal cancer: efficacy and circulating angiogenic biomarkers associated with therapeutic resistance. J Clin Oncol 2010;28:453-459.

(6) Fischer C, Jonckx B, Mazzone M, et al. Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. Cell 2007;131:463-475.

(7) Martinsson-Niskanen T, Riisbro R, Larsson L, et al. Monoclonal antibody TB-403:A first-in-human, phase I, double-blind, dose escalation study directed against placental growth factor in healthy male subjects. Clin Ther 2011;33:1142-1149.

(8) Lassen U, Nielsen DL, Sørensen M, et al. A phase I, dose-escalation study of TB-403, a monoclonal antibody directed against PlGF, in patients with advanced solid tumors. Br J Cancer 2012;106:678-684.