Targets in the microenvironment of rectal cancer

Tamas, Karin Rita

8

Discussion and Future Perspectives

Discussion and Future Perspectives

It is becoming increasingly clear over the last years that normal cells such as endothelial and immune cells, pericytes and cancer-associated fibroblasts, and other stromal cells substantially influence tumor growth, metastasis and sensitivity to cancer treatment through their interactions in the microenvironment with cancer cells (1-5). This insight opens new treatment possibilities as new targets are learned (6, 7).

CXCL12/CXCR4 axis as therapeutic target in rectal cancer

Potential cure is possible for patients with rectal cancer in case of limited metastatic spread to the liver or lungs. A reason for the limited chance to obtain this goal might be *de novo* or acquired resistance to anticancer treatment (2). In Chapter 4 we showed that rectal tumor and stromal cells express chemokine receptor CXCR4 and its ligand CXCL12 extensively at baseline, and that combined radiotherapy, chemotherapy and bevacizumab further upregulate expression of CXCL12. Stromal derived CXCL12 can directly stimulate proliferation and migration of CXCR4-expressing cancer cells. This specific prosurvival influence of stromal cells on tumor cells is thought to protect them from cytotoxic chemotherapy (8). CXCL12-mediated resistance to etoposide was described for small cell lung cancer cells when co-cultured with bone marrow stroma (9). In co-cultures of tumor and stromal cells and in murine models of chronic lymphocytic leukemia (10), acute myeloid leukemia (11, 12) and prostate cancer (8), inhibitors that block the binding pocket of CXCR4 (AMD3100, AMD3465, T140 analogs) sensitized cancer cells to chemotherapy (cytarabine, docetaxel) and the tyrosine kinase inhibitors sorafenib. Therefore, CXCL12/CXCR4 blockade could be tested in rectal cancer to rescue acquired resistance.

Although the CXCL12/CXCR4 ligand-receptor pair represents a potential clinical target in rectal cancer therapy, little is known about its role and dynamics in this disease. It

has a pleiotropic role in in several malignancies, and is ubiquitously present in both cancer and normal cells in various tissues (13). Therefore, in future studies in rectal cancer models the impact of CXCL12/CXCR4 blockade on tumor growth, survival and invasiveness, tumor cell mobilization and formation of metastasis, local stroma and tumor vasculature, mobilization of hematopoietic stem cells, and tumor infiltration with immune cells deserves attention (14).

Targeting PlGF in rectal cancer

Placental growth factor (PlGF) is a member of the VEGF family and is involved in bone marrow-derived cell activation, endothelial stimulation, pathologic angiogenesis and wound healing (7). In Chapter 5 we show that rectal tumors express PlGF extensively at diagnosis, and also after radiotherapy followed by bevacizumab, capecitabine and oxaliplatin treatment. A possible implication of this finding is that PlGF blockade might be of interest to test in rectal cancer patients. However, PlGF blockade resulted in antiangiogenic and antitumor effects in some models, but not in other (7). In this respect it would be of interest when a clinical study is considered to study PlGF expression by the tumor and to verify how much of the radioactive labeled antibody against PlGF gets into the tumor.

Molecular imaging to guide the use of antiangiogenic drugs

The administration of humanized monoclonal anti-PlGF antibody RO5323441 did not coincide with dose-limiting toxicity in early clinical trials (15, 16). Therefore, no optimal therapeutic dose is yet defined. Rational dosing might be achieved when tumor and normal body tissues uptake of the antibody is defined by ^{89}Zr-RO5323441 positron emission tomography (PET), using the tracer we developed in Chapter 6. By quantifying ^{89}Zr-RO5323441 tumor uptake we could get insight into target (PlGF) saturation by different doses of RO5323441. This could help defining the optimal therapeutic dose.

Furthermore, in a randomized trial in patients with recurrent glioblastoma, RO5323441 combined with bevacizumab did not improve the response rate compared to single agent bevacizumab (17). Vessel normalization is acknowledged for bevacizumab (18), and this could hamper the tumor penetration of large molecules such as antibodies (e.g., RO5323441 (19). ^{89}Zr-RO5323441 brain PET scanning could be used together with dynamic contrast-enhanced magnetic resonance imaging (MRI) to determine whether vessel normalization precluded RO5323441 penetration into the tumor. This might be of value for optimizing combined antiangiogenic treatment.

Fluorescent imaging in rectal cancer

The distance between the edge of the rectal tumor and the mesorectal fascia represents the circumferential resection margin (CRM), and constitutes the anatomical cornerstone for the feasibility of curative total mesorectal excision (TME). As presented in Chapter 2, MRI is the current preoperative examination of choice for establishing the CRM. However, there is room for improvement.

CXCR4 was found to facilitate cancer cell invasion in a mouse model of glioma and in colon carcinoma cells in culture (20, 21). CXCR4 antagonist TY14003 could be readily labeled with a fluorescent probe (carboxyfluorescein) (22). Fluorescent TY14003 specifically binds urothelial cancer cells and was used to image bladder cancer in mice by endoscopy. As shown in Chapter 4, CXCR4 is expressed at lower levels in epithelial cells of normal rectal crypts than in rectal cancer cells. Therefore, it could be envisioned that after injection, fluorescent-CXCR4 antagonist will most likely accumulate in the rectal tumor area. If CXCR4 is present at the invasive front (tumor rim) of rectal tumors, the fluorescent light emitted following light beam excitation would enable precise intraoperative CRM assessment during rectal tumor resection (23). Therefore, fluorescent CXCR4 antagonists constitute an option to be evaluated in this setting.

Conclusions

Advancing the understanding of tumor microenvironment-mediated disease progression and resistance to treatment in rectal cancer should help to define novel, rational and successful clinical approaches. New insights into angiogenic growth factors and chemokines as therapeutic targets in rectal cancer are presented in this thesis. Our clinical study shows that radical surgical treatment carried out after short-course radiotherapy, and capecitabine-oxaliplatin chemotherapy in combination with the VEGFA inhibitor bevacizumab is a feasible approach in primary metastasized rectal cancer. By examining the protein expression profiles of rectal cancer and stromal cells before and after therapy, we identified PlGF and the CXCL12/CXCR4 chemokine ligand-receptor pair as potential targets for improving rectal cancer therapy.

References

(1) Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-674.

(2) Meads MB, Gatenby RA, Dalton WS. Environment-mediated drug resistance: a major contributor to minimal residual disease. Nature Rev Cancer 2009;9:665-674.

(3) Ebos JM, Lee CR, Kerbel RS. Tumor and host-mediated pathways of resistance and disease progression in response to antiangiogenic therapy. Clin Cancer Res 2009;15:5020-5025.

(4) Pietras K, Ostman A. Hallmarks of cancer: interactions with the tumor stroma. Exp Cell Res 2010;316:1324-1331.

(5) Olson OC, Joyce JA. Microenvironment-mediated resistance to anticancer therapies. Cell Res 2013;23:179-181.

(6) Lappano R, Maggiolini M. G protein-coupled receptors: novel targets for drug discovery in cancer. Nat Rev Drug Discov 2011;10:47-60.

(7) 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.

(8) Domanska UM, Timmer-Bosscha H, Nagengast WB, et al. CXCR4 inhibition with AMD3100 sensitizes prostate cancer to docetaxel chemotherapy. Neoplasia 2012;14:709-718.

(9) Hartmann TN, Burger JA, Glodek A, Fujii N, Burger M. CXCR4 chemokine receptor and integrin signaling co-operate in mediating adhesion and chemoresistance in small cell lung cancer (SCLC) cells. Oncogene 2005;24:4462-4471.

(10) Burger M, Hartmann T, Krome M, et al. Small peptide inhibitors of the CXCR4 chemokine receptor (CD184) antagonize the activation, migration, and antiapoptotic responses of CXCL12 in chronic lymphocytic leukemia B cells. Blood 2005;106:1824-1830.

(11) Zeng Z, Shi YX, Samudio IJ, et al. Targeting the leukemia microenvironment by CXCR4 inhibition overcomes resistance to kinase inhibitors and chemotherapy in AML. Blood 2009;113:6215-6224.

(12) Nervi B, Ramirez P, Rettig MP, et al. Chemosensitization of acute myeloid leukemia (AML) following mobilization by the CXCR4 antagonist AMD3100. Blood 2009;113:6206-6214.

(13) Domanska UM, Kruizinga RC, Nagengast WB, et al. A review on CXCR4/CXCL12 axis in oncology: no place to hide. Eur J Cancer 2013;49:219-230.

(14) Francia G, Kerbel RS. Raising the bar for cancer therapy models. Nat Biotechnol. 2010;28:561-562.

(15) 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.

(16) 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

(17) Lassen U, Chinot O, McBain C, et al. Phase I study of anti-PlGF monoclonal antibody (mAb) RO5323441 (RO) and anti-VEGF mab bevacizumab (BV) in patients with recurrent glioblastoma (GBM). J Clin Oncol 2013;31(Suppl 4):2092 (Abstr).

(18) Jain RK. Normalizing tumor microenvironment to treat cancer: bench to bedside to biomarkers. J Clin Oncol 2013;31:2205-2218.

(19) Arjaans M, Oude Munnink TH, Oosting SF, et al. Bevacizumab-induced normalization of blood vessels in tumors hampers antibody uptake. Cancer Res 2013;73:3347-3355.

(20) Kirkpatrick ND, Jain RK. In vivo vessel co-option by glioma cells following chronic anti-angiogenic treatment. Cancer Res 2011;70:368 (abstract).

(21) Heckmann D, Maier P, Laufs S, et al. The disparate twins: a comparative study of CXCR4 and CXCR7 in SDF-1α-induced gene expression, invasion and chemosensitivity of colon cancer. Clin Cancer Res 2014;20:604-616.

(22) Weiss ID, Jacobson O. Molecular Imaging of Chemokine Receptor CXCR4.
Theranostics 2013;3:76–84.

(23) Van Dam GM, Themelis G, Crane LM, et al. Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-α targeting: first in-human results. Nat Med 2011;17:1315-1319.