Differential response to EGFR- and VEGF-targeted therapies in patient-derived tumor tissue xenograft models of colon carcinoma and related metastases

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Abstract. Heterogeneity in primary tumors and related metastases may result in failure of antitumor therapies, particularly in targeted therapies for the treatment of cancer. In this study, patient-derived tumor tissue (PDTT) xenograft models of colon carcinoma with lymphatic and hepatic metastases were used to evaluate the response to EGFR- and VEGF-targeted therapies. Our results showed that primary colon carcinoma and its corresponding lymphatic and hepatic metastases have a different response rate to anti-EGFR (cetuximab) and anti-VEGF (bevacizumab) therapies. However, the underlying mechanism of these types of phenomenon is still unclear. To investigate whether such phenomena may result from the heterogeneity in primary colon carcinoma and related metastases, we compared the expression levels of cell signaling pathway proteins using immunohistochemical staining and western blotting, and the gene status of *KRAS* using pyrosequencing in the same primary colon carcinoma and its corresponding lymphatic and hepatic metastatic tissues which were used for establishing the PDTT xenograft models. Our results showed that the expression levels of EGFR, VEGF, Akt/pAkt, ERK/pERK, MAPK/pMAPK, and mTOR/pmTOR were different in primary colon carcinoma and matched lymphatic and hepatic metastases although the *KRAS* gene status in all cases was wild-type. Our results indicate that the heterogeneity in primary colon carcinoma and its corresponding lymphatic and hepatic metastases may result in differences in the response to dual-inhibition of EGFR and VEGF.

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Introduction

Intratumor heterogeneity is one of the recognized characteristics of human tumors, and occurs on multiple levels, such as the genetic, protein and macroscopic level in a wide range of tumors, including breast, colorectal (CRC), non-small cell lung (NSCLC), prostate, ovarian, pancreatic, gastric, and brain cancer and renal clear cell carcinoma (1). In recent years, many studies have focused on the heterogeneity found in primary tumors and related metastases with the consideration that evaluation of metastatic rather than primary sites could be of clinical relevance. Numerous reports have evaluated the genetic heterogeneity in primary tumors and corresponding metastases in a range of solid tumors such as breast cancer (2-9), CRC (10-13) and NSCLC (14,15). Heterogeneity in primary tumors and related metastases may result in the failure of antitumor therapies, particularly in targeted therapies for the treatment of cancer (16).

However, without a suitable tumor model we cannot elucidate whether such heterogeneity results in different responses to anticancer therapy. In a previous study, we successfully established the patient-derived tumor tissue (PDTT) xenograft models of colon carcinoma with lymphatic and hepatic metastases (17). The ideal biological characteristics of such PDTT xenograft models, as previously described (17), confirmed our hypothesis that such PDTT models would help us investigate the underlying mechanism of the differences in heterogeneity-related anticancer therapy response in primary colon carcinoma and its corresponding lymphatic and hepatic metastases.

In this study, PDTT xenograft models of colon carcinoma with lymphatic and hepatic metastases were used to evaluate the response to EGFR- and VEGF-targeted therapies. We also investigated heterogeneity in primary colon carcinoma tissue

and its corresponding lymphatic and hepatic metastatic tissues from the same metastatic colon carcinoma patient focusing on the cell signaling pathway proteins.

Materials and methods

Reagents and drugs. Anti-Akt, anti-ERK, anti-MAPK and anti-mTOR antibodies, and phosphorylation-specific antibodies against Akt (Ser[308] and Ser[473]), ERK (Thr[202]/Tyr[204]), MAPK (Thr[180]/Tyr[182]) and mTOR (Ser[2448]) as well as the antibody against cleaved caspase-3 were purchased from Cell Signaling Technology Inc. (Cell Signaling, Beverly, MA). The antibodies against VEGF and EGFR were purchased from Epitomics Inc. (Burlingame, CA). The antibody against GAPDH was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Horseradish peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Chemiluminescent detection system was purchased from Amersham Pharmacia Biotech (Arlington Heights, IL). Bevacizumab (Avastin®) was purchased from Roche, Inc. (Roche, USA). Cetuximab was purchased from Merck, Inc. (Merck, Darmstadt, Germany).

Patient and tissue samples. Tumor specimens were obtained at initial surgery from a 40-year-old female colon carcinoma patient with lymphatic and hepatic metastases. Prior written informed consent was obtained from the patient and the study received approval from the Ethics Board of the First Affiliated Hospital, College of Medicine, Zhejiang University. The patient had not received chemotherapy or radiation therapy before surgery. The histological type was determined according to WHO criteria. The tumor was diagnosed as mucinous adenocarcinoma (T3N2M1). The tumor samples of colon carcinoma with lymphatic and hepatic metastases were put into medium immediately after surgical resection under sterile conditions and transported without delay to the animal facility.

Establishment of xenografts and treatment protocol. Four- to six-week-old female BALB/c nude mice purchased from Slaccas (Slaccas Laboratory Animal, Shanghai, China) were housed in a barrier facility and acclimated to 12-h light/12-h dark cycles for at least three days before use. The use of experimental animals adhered to the 'Principles of Laboratory Animal Care' (NIH publication No. 85-23, revised in 1985). All experiments were approved by the Institutional Animal Care and Use Committee of Zhejiang University [approval ID: SYXK(ZHE)2005-0072]. The method to establish the PDTT xenograft models of human colon carcinoma with lymphatic and hepatic metastases were described previously (1,17-20).

Xenografts from this second mouse-to-mouse passage were allowed to grow to a size of 200 mm³, at which time mice were randomized into the following three cohorts: cohort of primary colon carcinoma xenografts, cohort of lymphatic metastasis xenografts, and cohort of hepatic metastasis xenografts. In each cohort, xenografts were randomized into four groups with 10 mice in each group: (a) control (saline 100 μl i.v. + 200 μl i.p., twice per week); (b) bevacizumab (Avastin), 10 mg/kg in 100 μl, i.v., twice per week; (c) cetuximab, 10 mg/kg in 200 μl, i.p., twice per week; (d) cetuximab, 10 mg/kg in 200 μl, i.p., twice per week + bevacizumab, 10 mg/kg in

100 μl, i.v., twice per week. Mice were treated for 21 days, monitored twice per week for signs of toxicity, and were weighed once a week. Tumor size was evaluated twice a week by caliper measurements using the following formula: tumor volume = (length x width²)/2. Relative tumor growth inhibition (TGI) was calculated by relative tumor growth of treated mice divided by relative tumor growth of control mice (T/C). Experiments were terminated on day 30. This experiment was repeated twice with similar results.

DNA extraction and mutation analyses. DNA was extracted from paraffin-embedded samples of colon carcinoma with lymphatic and hepatic metastases. For every tumor tissue, 10-μm sections were prepared, and an additional representative 2-μm section was deparaffinized, stained with haematoxylin and eosin, and analyzed for detailed morphology. Regions of tumor tissue were marked, and this tissue was extracted with 0.2 M sodium hydroxide in 1 mM edetic acid and neutralized with 100 mM Tris-TE (pH 6.5). After extraction, DNA was purified with Qiagen PCR purification kit (Qiagen, Hilden, Germany). *KRAS* gene exon 1 was analysed at codons 12 and 13 with pyrosequencing using a previously described assay which has been shown to be of greater sensitivity (21).

Immunohistochemistry. Selected tumor specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections (5 μm) were cut, dewaxed, rehydrated, and subjected to antigen retrieval. After blocking endogenous peroxidase activity, the sections were incubated with the primary antibodies against EGFR (1:100) and VEGF (1:100) overnight at 4˚C. Immunohistochemistry was performed using the streptavidin-biotin peroxidase complex method (Lab Vision, Fremont, CA). The slides were examined and images were captured using an Olympus BX60 (Olympus, Japan). Sections known to stain positively were incubated in each batch and negative controls were also prepared by replacing the primary antibody with preimmune sera.

Western blotting. Protein expression profiles were analyzed by western blotting as previously described (22-24). Briefly, lysates for immunoblotting were prepared by adding lysis buffer [50 mM Tris-HCl (pH 7.4), 1% Nonidet P-40, 0.5% sodium deoxycholate, 150 mM NaCl, 0.02% sodium azide, and 0.1% SDS] containing protease and phosphatase inhibitors (Sigma, St. Louis, MO) to the tumor tissue homogenized in fluid nitrogen. After centrifugation at 15,000 rpm at 4˚C for 10 min, the supernatants were collected, and the protein concentration was determined using Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). Protein extracts of tumor lysates (30 μg) were added to a loading buffer [10 mmol/l Tris-HCl (pH 6.8), 1% SDS, 25% glycerol, 0.1 mmol/l mercaptoethanol, and 0.03% bromophenol blue], boiled, and separated on 8-12% (w/v) polyacrylamide gels in the presence of SDS. Molecular weights of the immunoreactive proteins were estimated based on the relative migration with colored molecular weight protein markers (Amersham Pharmacia Biotech, Piscataway, NJ). Following electrophoresis, the protein blots were electro-transferred to PVDF membranes (Millipore, Billerica, MA). The membranes were then blocked at room temperature with 5% nonfat milk in TBS [10 mmol/l Tris-HCl
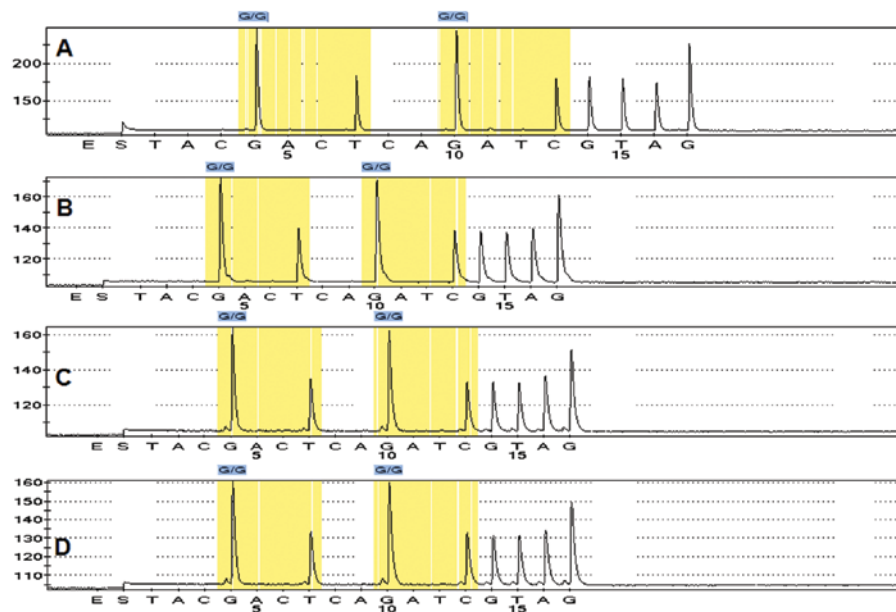
Figure 1. *KRAS* mutation analysis by pyrosequencing. (A) Sequence data of *KRAS* in exon 1 at codons 12 and 13 in standard wild-type sample, and (B) in tumor tissues of primary colon cancer, (C) lymphatic metastasis, and (D) hepatic metastasis.

(pH 7.5), 0.5 mol/l NaCl, and 0.05% (v/v) Tween-20] buffer for 1 h. The primary antibodies were diluted at 1:1,000 and the membranes were incubated with primary antibodies overnight at 4°C. The antibodies tested were anti-Akt, anti-ERK, anti-MAPK, anti-mTOR antibodies, anti-EGFR, anti-VEGF, anti-cleaved caspase-3, and phosphorylation-specific antibodies against Akt (Ser^{308} and Ser^{473}), ERK (Thr^{202}/Tyr^{204}), MAPK (Thr^{180}/Tyr^{182}) and mTOR (Ser^{2448}). The following day, the membranes were washed and incubated for 1 h at room temperature with rabbit immunoglobulin G-horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology), at a final dilution of 1:5,000. After washing thrice with TBS, antibody binding was visualized using enhanced chemiluminescence detection system (SuperSignal West Pico, Pierce) as described by the manufacturer and autoradiography. To show equal protein loading, the blots were stripped and reprobed for GAPDH. This experiment was repeated three times with similar results.

Statistical analysis. Drug sensitivity data are presented as the mean ± SEM and analyzed by SPSS 16.0 software. Difference among mean of the groups is determined with one-way ANOVA. Comparison is considered to be statistically significant at p<0.05.

Results

PDTT xenograft models of primary colon carcinoma and related metastases have different response rates to dual-inhibition of EGFR and VEGF. It is necessary to ascertain the molecular basis for the response to cetuximab in metastatic colon carcinoma. For this purpose, we assessed the mutation status of the *KRAS* gene exon 1 at codons 12 and 13 in primary colon carcinoma and its lymphatic and hepatic metastases. Our results revealed that the *KRAS* gene status in the three tumor sites are all wild-type (Fig. 1).

We subsequently evaluated the therapy response of cetuximab in combination with bevacizumab in the primary colon carcinoma and its corresponding lymphatic and hepatic metastases using the PDTT xenograft models. Our results showed that all xenografts of primary colon carcinoma and corresponding lymphatic and hepatic metastases in nude mice responded to the dual-inhibition of EGFR and VEGF (Fig. 2A-C). However, dual-inhibition of EGFR and VEGF resulted in significantly different relative TGI in xenografts of primary colon carcinoma (22.2%) and corresponding lymphatic (9.6%) and hepatic metastasis (9.9%) (Fig. 2A-D). Our results demonstrate that primary colon carcinoma and its corresponding lymphatic and hepatic metastases have different response rates to anti-EGFR and anti-VEGF therapies.

Heterogeneity in primary colon carcinoma and its corresponding lymphatic and hepatic metastases. Immuno-histochemical staining revealed that EGFR and VEGF expression levels in primary colon carcinoma tissue are different from those in its lymphatic and hepatic metastases. Our findings reveal that the expression levels of EGFR (Fig. 3) and VEGF (Fig. 4) in metastatic tissues were higher than those in primary colon carcinoma tissue.

In this study, the expression levels of the EGFR and VEGF downstream signaling pathway proteins were further determined using western blotting. The expression levels of EGFR, VEGF, pAkt, and mTOR in metastatic tissues were found to be higher than those in primary colon carcinoma tissue (Fig. 5), while the expression levels of Akt, ERK, MAPK, and pmTOR in primary colon carcinoma tissue were higher than those in metastatic tissues (Fig. 5). Hepatic metastasis had the highest expression levels of pERK and pMAPK (Fig. 5). Our results indicate that the heterogeneity of EGFR- and VEGF-related signaling pathway proteins exist in primary colon carcinoma and its corresponding lymphatic and hepatic metastases. Furthemore, our findings indicate that heterogeneity in primary
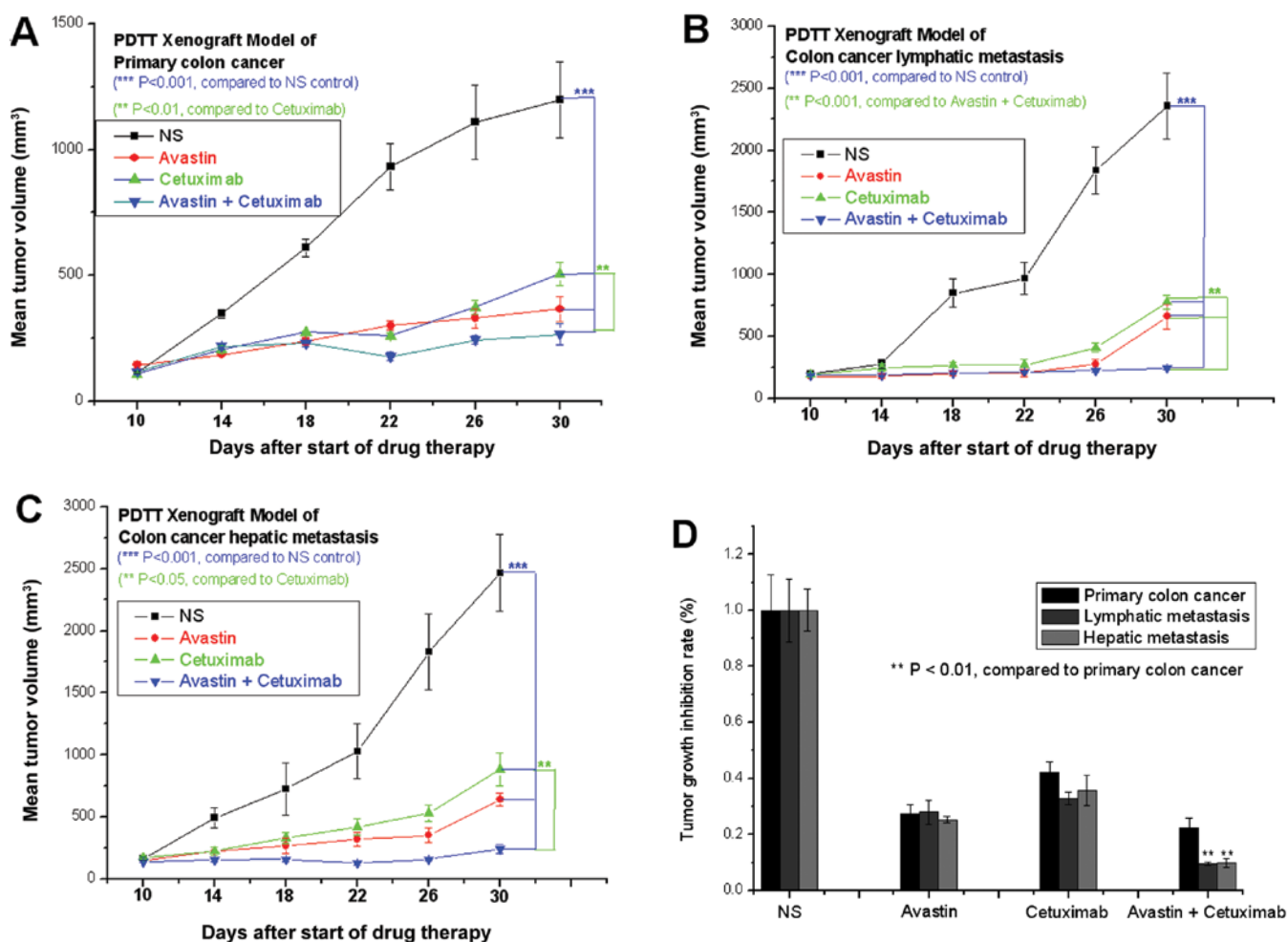
Figure 2. Response curve of cetuximab, bevacizumab (Avastin), and cetuximab + bevacizumab in PDTT xenograft models of (A) primary colon carcinoma, (B) lymphatic metastasis, and (C) hepatic metastasis, and (D) the response rate to cetuximab, bevacizumab, and cetuximab + bevacizumab in PDTT xenograft models of three tumor sites. Ten mice per group were treated with the corresponding agent. Data shown are the means ± SEM. The differences between control tumor volumes, cetuximab-treated, bevacizumab-treated and cetuximab + bevacizumab-treated tumor volumes and the response rates to the same treatment in xenografts of three tumor sites were analyzed by one-way ANOVA. Experiments were repeated at least twice with similar results.
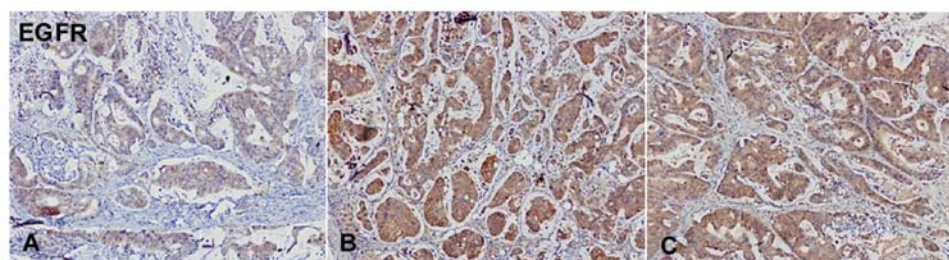

Figure 3. Immunohistochemical staining of EGFR. (A) Primary colon carcinoma, (B) lymphatic metastasis (C) hepatic metastasis. Original magnification, x100.
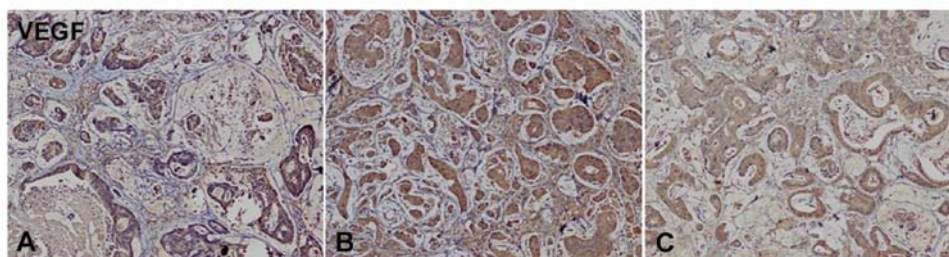

Figure 4. Immunohistochemical staining of VEGF. (A) Primary colon carcinoma, (B) lymphatic metastasis, (C) hepatic metastasis. Original magnification, x100.
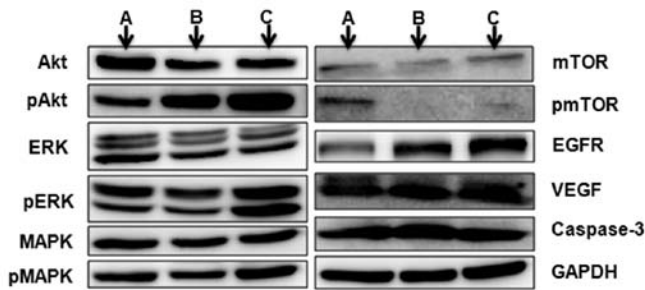
Figure 5. Immunoblotting data of the proteins Akt, pAkt (Ser^{308} and Ser^{473}), ERK, pERK (Thr^{202}/Tyr^{204}), MAPK, pMAPK (Thr^{180}/Tyr^{182}), mTOR, pmTOR (Ser^{2448}), EGFR, VEGF, cleaved caspase-3, and GAPDH (as loading control) of (A) primary colon carcinoma and its corresponding (B) lymphatic and (C) hepatic metastases.

colon carcinoma and its corresponding lymphatic and hepatic metastases may partially make contribute to differences in response to anti-EGFR or anti-VEGF targeted therapies.

Discussion

The main purpose of investigating the heterogeneity in primary tumors and their corresponding metastases is to evaluate the effects of such heterogeneity on the efficacy of anticancer therapy and cancer patients' prognosis. As we have previously reported (1,17,19), the PDTT xenograft model which has a sound establishing method and a retained similarity to the corresponding original donor tumors in histological presentation and biological behavior, such as protein expression, tumor biomarker status, and genomic and genetic status, has the potential to be a good strategy to achieve our purpose.

In our previous study, we established the PDTT xenograft models of colon carcinoma with lymphatic and hepatic metastases (17). The biological characteristics of such PDTT xenograft models, as previously described (17), confirmed our belief that such PDTT models would aid in our investigation of the underlying mechanism of heterogeneity-related anticancer therapy response differences in primary colon carcinoma and its corresponding lymphatic and hepatic metastases. Based on this hypothesis and considering that the *KRAS* gene status in the three tumor sites are all wild-type, the drug sensitivity of bevacizumab (Avastin) in combination with cetuximab in the primary colon carcinoma and its corresponding lymphatic and hepatic metastases was evaluated in this study using the PDTT xenograft models.

In the present study we also investigated the heterogeneity in primary colon carcinoma and its corresponding lymphatic and hepatic metastases focusing on the cell signaling pathway proteins using immunohistochemical staining and western blotting. We found that the expression levels of EGFR, VEGF, Akt/pAkt, ERK/pERK, MAPK/pMAPK, and mTOR/pmTOR were different in primary colon carcinoma and matched lymphatic and hepatic metastases, although the *KRAS* gene status in all was wild-type.

With regard to CRC, the therapeutic benefit of EGFR-targeted monoclonal antibodies such as cetuximab and panitumumab has been established in various studies (25-27). Notably, no correlation was observed between the expression

levels of EGFR and therapeutic success (25-27), and even patients with tumors apparently lacking EGFR expression responded to antibody therapy in up to 25% of the cases (28-30). In our study, xenografts of primary colon carcinoma and its corresponding lymphatic and hepatic metastases all responded to cetuximab (Fig. 2A-C). However, no significant difference could be observed in these groups (Fig. 2D) although they have different expression levels of EGFR (Fig. 3 and 5).

Across a wide range of human tumors and/or cell lines, expression of VEGF has been shown to lead to the development and maintenance of a vascular network that promotes tumor growth and metastasis. Moreover, a large and growing body of evidence indicates that both VEGF gene expression and production are associated closely with poor prognosis (31-35). However, no correlation was observed between the expression levels of VEGF and clinical outcomes of VEGF targeted therapy (36). Our findings show that primary colon carcinoma and its corresponding lymphatic and hepatic metastases have different expression levels of VEGF (Fig. 4 and 5), but they all responded to bevacizumab (Fig. 2A-C), and no significant difference was observed in these groups (Fig. 2D).

Moreover, xenografts of primary colon carcinoma and its corresponding lymphatic and hepatic metastases have different response rates to treatment of bevacizumab in combination with cetuximab (Fig. 2D) although all xenografts responded to the dual-inhibition of EGFR and VEGF (Fig. 2A-C). Our results demonstrated that dual-inhibition of EGFR and VEGF could result in significantly different response rates in primary colon carcinoma and corresponding metastases if the EGFR and VEGF expression levels are different in these tumors. Our results indicate that heterogeneity in primary colon carcinoma and its corresponding lymphatic and hepatic metastases may result in differences in response to dual-inhibition of EGFR and VEGF.

In this study, we investigated heterogeneity in primary colon carcinoma and its corresponding lymphatic and hepatic metastases focusing on the cell signaling pathway proteins, and we found that the levels of EGFR, VEGF, Akt/pAkt, ERK/pERK, MAPK/pMAPK, and mTOR/pmTOR were different in primary colon carcinoma and matched lymphatic and hepatic metastases. Furthermore, with the help of PDTT xenograft models, we demonstrated that such heterogeneity would result in different responses to anti-EGFR and anti-VEGF targeted therapies. The PDTT xenograft model could be a good *in vivo* tool to examine whether the primary tumors and corresponding metastases have different responses to the same anticancer drugs.

Acknowledgements

References

1. Jin KT, He KF, Teng F, Han N, Li GL, Xu ZZ and Teng LS: Heterogeneity in primary tumors and corresponding metastases: could it provide us with any hints to personalize cancer therapy? Pers Med 8: 175-182, 2011.
2. Gong Y, Booser DJ and Sneige N: Comparison of HER-2 status determined by fluorescence in situ hybridization in primary and metastatic breast carcinoma. Cancer 103: 1763-1769, 2005.
3. Gancberg D, Di Leo A, Cardoso F, Rouas G, Pedrocchi M, Paesmans M, Verhest A, Bernard-Marty C, Piccart MJ and Larsimont D: Comparison of HER-2 status between primary breast cancer and corresponding distant metastatic sites. Ann Oncol 13: 1036-1043, 2002.
4. Regitnig P, Schippinger W, Lindbauer M, Samonigg H and Lax SF: Change of HER-2/neu status in a subset of distant metastases from breast carcinomas. J Pathol 203: 918-926, 2004.
5. Bozzetti C, Personeni N, Nizzoli R, Guazzi A, Flora M, Bassano C, Negri F, Martella E, Naldi N, Franciosi V and Cascinu S: HER-2/neu amplification by fluorescence in situ hybridization in cytologic samples from distant metastatic sites of breast carcinoma. Cancer 99: 310-315, 2003.
6. Tanner M, Järvinen P and Isola J: Amplification of HER-2/neu and topoisomerase IIalpha in primary and metastatic breast cancer. Cancer Res 61: 5345-5348, 2001.
7. Tapia C, Savic S, Wagner U, *et al*: HER2 gene status in primary breast cancers and matched distant metastases. Breast Cancer Res 9: R31, 2007.
8. Akcakanat A, Sahin A, Shaye AN, Velasco MA and Meric-Bernstam F: Comparison of Akt/mTOR signaling in primary breast tumors and matched distant metastases. Cancer 112: 2352-2358, 2008.
9. Wu JM, Fackler MJ, Halushka MK, *et al*: Heterogeneity of breast cancer metastases: comparison of therapeutic target expression and promoter methylation between primary tumors and their multifocal metastases. Clin Cancer Res 14: 1938-1946, 2008.
10. Baldus SE, Schaefer KL, Engers R, Hartleb D, Stoecklein NH and Gabbert HE: Prevalence and heterogeneity of KRAS, BRAF, and PIK3CA mutations in primary colorectal adenocarcinomas and their corresponding metastases. Clin Cancer Res 16: 790-799, 2010.
11. Molinari F, Martin V, Saletti P, De Dosso S, Spitale A, Camponovo A, Bordoni A, Crippa S, Mazzucchelli L and Frattini M: Differing deregulation of EGFR and downstream proteins in primary colorectal cancer and related metastatic sites may be clinically relevant. Br J Cancer 100: 1087-1094, 2009.
12. Scartozzi M, Bearzi I, Berardi R, Mandolesi A, Fabris G and Cascinu S: Epidermal growth factor receptor (EGFR) status in primary colorectal tumors does not correlate with EGFR expression in related metastatic sites: implications for treatment with EGFR-targeted monoclonal antibodies. J Clin Oncol 22: 4772-4778, 2004.
13. Scartozzi M, Bearzi I, Berardi R, Mandolesi A, Pierantoni C and Cascinu S: Epidermal growth factor receptor (EGFR) downstream signalling pathway in primary colorectal tumours and related metastatic sites: optimising EGFR-targeted treatment options. Br J Cancer 97: 92-97, 2007.
14. Sasatomi E, Finkelstein SD, Woods JD, Bakker A, Swalsky PA, Luketich JD, Fernando HC and Yousem SA: Comparison of accumulated allele loss between primary tumor and lymph node metastasis in stage II non-small cell lung carcinoma: implications for the timing of lymph node metastasis and prognostic value. Cancer Res 62: 2681-2689, 2002.
15. Park S, Holmes-Tisch AJ, Cho EY, *et al*: Discordance of molecular biomarkers associated with epidermal growth factor receptor pathway between primary tumors and lymph node metastasis in non-small cell lung cancer. J Thorac Oncol 4: 809-815, 2009.
16. Li Z, Jin K, Lan H and Teng L: Heterogeneity in primary colorectal cancer and its corresponding metastases: a potential reason of EGFR-targeted therapy failure? Hepatogastroenterology 58: 411-416, 2011.
17. Jin K, Li G, Cui B, *et al*: Assessment of a novel VEGF targeted agent using patient-derived tumor tissue xenograft models of colon carcinoma with lymphatic and hepatic metastases. PLoS One 6: e28384, 2011.
18. Jin K, Teng L, Shen Y, He K, Xu Z and Li G: Patient-derived human tumour tissue xenografts in immunodeficient mice: a systematic review. Clin Transl Oncol 12: 473-480, 2010.
19. Jin KT, He KF, Li GL and Teng LS: Personalized cancer therapy using a patient-derived tumor tissue xenograft model: a translational field worthy of exploring further? Pers Med 7: 597-606, 2010.
20. Jin K, He K, Han N, Li G, Wang H, Xu Z, Jiang H, Zhang J and Teng L: Establishment of a PDTT xenograft model of gastric carcinoma and its application in personalized therapeutic regimen selection. Hepatogastroenterology 58: 1814-1822, 2011.
21. Ogino S, Kawasaki T, Brahmandam M, Yan L, Cantor M, Namgyal C, Mino-Kenudson M, Lauwers GY, Loda M and Fuchs CS: Sensitive sequencing method for KRAS mutation detection by Pyrosequencing. J Mol Diagn 7: 413-421, 2005.
22. Huynh H, Chow PK, Ooi LL and Soo KC: A possible role for insulin-like growth factor-binding protein-3 autocrine/paracrine loops in controlling hepatocellular carcinoma cell proliferation. Cell Growth Differ 13: 115-122, 2002.
23. Rubio-Viqueira B, Jimeno A, Cusatis G, *et al*: An in vivo platform for translational drug development in pancreatic cancer. Clin Cancer Res 12: 4652-4661, 2006.
24. Perez-Soler R, Kemp B, Wu QP, Mao L, Gomez J, Zeleniuch-Jacquotte A, Yee H, Lee JS, Jagirdar J and Ling YH: Response and determinants of sensitivity to paclitaxel in human non-small cell lung cancer tumors heterotransplanted in nude mice. Clin Cancer Res 6: 4932-4938, 2000.
25. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I and Van Cutsem E: Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351: 337-345, 2004.
26. Saltz LB, Meropol NJ, Loehrer PJ Sr, Needle MN, Kopit J and Mayer RJ: Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol 22: 1201-1208, 2004.
27. Amado RG, Wolf M, Peeters M, *et al*: Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 26: 1626-1634, 2008.
28. Cappuzzo F, Varella-Garcia M, Finocchiaro G, *et al*: Primary resistance to cetuximab therapy in EGFR FISH-positive colorectal cancer patients. Br J Cancer 99: 83-89, 2008.
29. Moroni M, Veronese S, Benvenuti S, Marrapese G, Sartore-Bianchi A, Di Nicolantonio F, Gambacorta M, Siena S and Bardelli A: Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to anti-EGFR treatment in colorectal cancer: a cohort study. Lancet Oncol 6: 279-286, 2005.
30. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, Zanon C, Moroni M, Veronese S, Siena S and Bardelli A: Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res 67: 2643-2648, 2007.
31. Foekens JA, Peters HA, Grebenchtchikov N, Look MP, Meijer-van Gelder ME, Geurts-Moespot A, van der Kwast TH, Sweep CG and Klijn JG: High tumor levels of vascular endothelial growth factor predict poor response to systemic therapy in advanced breast cancer. Cancer Res 61: 5407-5414, 2001.
32. Gasparini G, Toi M, Gion M, *et al*: Prognostic significance of vascular endothelial growth factor protein in node-negative breast carcinoma. J Natl Cancer Inst 89: 139-147, 1997.
33. Konecny GE, Meng YG, Untch M, *et al*: Association between HER-2/neu and vascular endothelial growth factor expression predicts clinical outcome in primary breast cancer patients. Clin Cancer Res 10: 1706-1716, 2004.
34. Linderholm B, Grankvist K, Wilking N, Johansson M, Tavelin B and Henriksson R: Correlation of vascular endothelial growth factor content with recurrences, survival, and first relapse site in primary node-positive breast carcinoma after adjuvant treatment. J Clin Oncol 18: 1423-1431, 2000.
35. Gasparini G, Toi M, Miceli R, *et al*: Clinical relevance of vascular endothelial growth factor and thymidine phosphorylase in patients with node-positive breast cancer treated with either adjuvant chemotherapy or hormone therapy. Cancer J Sci Am 5: 101-111, 1999.
36. Verschraegen CF, Arias-Pulido H, Lee SJ, *et al*: Phase IB study of the combination of docetaxel, gemcitabine, and bevacizumab in patients with advanced or recurrent soft tissue sarcoma: the Axtell regimen. Ann Oncol 23: 785-790, 2012.