Tumor calcification as a prognostic factor in cetuximab plus chemotherapy-treated patients with metastatic colorectal cancer

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This study aimed to explore the correlation between survival and tumor calcification in patients with metastatic colorectal cancer who received cetuximab combined with chemotherapy. The study was a single-center retrospective analysis that enrolled 111 patients who had received therapy between April 2011 and October 2016. Tumor calcification and treatment efficacy were evaluated independently by radiologists on the basis of computed tomography scans. Clinical characteristics and follow-up data were collected from electronic medical records. Correlations between tumor calcification and clinical characteristics, tumor response rate, and patient survival were analyzed. Among the 111 enrolled patients, 27 had tumor calcification [27/111 (24.3%)]. The median progression-free survival was significantly longer for patients with tumor calcification than for those without calcification (9.3 vs. 6.2 months, $P = 0.022$). Patients with tumor calcification also had a higher objective response rate (55.6 vs. 31%, $P = 0.021$) and better overall survival (21.9 vs. 16.5 months, $P = 0.084$). The correlation between calcification features and prognosis showed that patients with an increasing number of calcifications after treatment had a significantly longer median overall survival (22.9 vs. 9.1 months, $P = 0.033$). Simultaneously, new liver metastases and multiple calcifications also showed a trend toward better overall survival. There were also no significant correlations between clinical characteristics (sex, age, gene mutation, primary tumor location, pathological type, blood test result) and survival (Supplementary Table 1, Supplemental digital content 1, *http://links.lww.com/ACD/A280*). Tumor calcification is associated with a better treatment outcome and is a potential prognostic marker. *Anti-Cancer Drugs* **30:195–200 Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc.**

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Background

Colorectal cancer (CRC) is the second most common type of cancer and the fourth leading cause of cancer-associated death worldwide [1]. Cetuximab has shown clinically important improvements in overall survival (OS) and progression-free survival (PFS) among *KRAS* wide-type patients with metastastic CRC (mCRC) [2–4], both as monotherapy and in combination with chemotherapy. In the course of cetuximab treatment, early tumor shrinkage [5–7] and the appearance of tumor calcification are often observed by scrupulous clinicians. Abdominal neoplastic calcification is reported commonly in adenocarcinoma, for example, colorectal and ovarian adenocarcinoma [8], and Ko *et al.* [9] reported that calcification is most commonly seen in ovarian mucinous adenocarcinoma and predicts a poor prognosis. Calcification was present in ~12–27% of patients with CRC liver metastasis [10,11].

To date, the predictive or prognostic value of tumor calcification in mCRC was unclear [12]. Only two opposing reports have been published on the correlation between tumor calcification and patient prognosis in colorectal adenocarcinoma. Easson *et al.* [13] reported that calcification of liver metastasis was associated with longer survival, irrespective of the number of metastases and tumor differentiation. Another study found that the calcification status in liver metastases was not fixed, and calcification in early metastases did not influence patient prognosis [14]. These previous reports involved patients who received only chemotherapy. In the modern treatment regimen, targeted drugs are combined with chemotherapy as the standard of care for mCRC. To date, there have been no relevant studies

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on the relationship between targeted drugs and tumor calcification in mCRC.

The aim of this retrospective study was to explore the relationship between tumor calcification status and tumor characteristics and survival in mCRC patients treated with cetuximab and chemotherapy.

Materials and methods

Materials

We retrospectively analyzed mCRC patients who received cetuximab at West China Hospital from April 2011 to October 2016. The inclusion criteria were as follows: (i) pathological confirmation of colorectal adenocarcinoma; (ii) unresectable stage IV disease; (iii) wild-type *KRAS* gene; (iv) at least one measurable lesion according to RECIST 1.1; (v) cetuximab combined with chemotherapy (based on oxaliplatin or irinotecan) was administered as first-line or second-line chemotherapy; and (vi) at least one computed tomography (CT)-based evaluation of treatment efficacy and all imaging data were available for retrospective analysis. The following exclusion criteria were as follows: (i) the patient had undergone surgery for metastases, radiotherapy, or local treatment on measurable lesions before the first response evaluation; (ii) not evaluated by CT during cetuximab combination treatment; or (iii) complete clinical material needed for the study was lacking.

The chemotherapy regimens in our study were mFOLFOX6 and FOLFIRI. Both doses of chemotherapy and cetuximab were consistent with NCCN guidelines. The mFOLFOX 6 protocol [oxaliplatin 85 mg/m^2 intravenously (i.v.) on day 1, leucovorin 400 mg/m^2 i.v. on day 1, 5-fluorouracil (5-FU) 400 mg/m^2 i.v. bolus on day 1, then total 2400 mg/m^2 over 46–48 h i.v. continuous infusion, every 2 weeks] or the FOLFIRI protocol (irinotecan 180 mg/m^2 i.v. over 30–90 min on day 1, leucovorin 400 mg/m^2 i.v. on day 1, 5-FU 400 mg/m^2 i.v. bolus on day 1, then total 2400 mg/m^2 over 46–48 h i.v. continuous infusion, every 2 weeks) was combined with cetuximab (400 mg/m^2 i.v. over 2 h first infusion then 250 mg/m^2 i.v. over 60 min weekly or 500 mg/m^2 i.v. over 2 h on day 1, every 2 weeks) as the first-line or second-line treatment for mCRC.

This study was approved by the Medical Ethical Committee of West China Hospital of Sichuan University (Chengdu, China), and all patients in this research provided informed consent.

Working methods

According to the standard treatment strategy, each patient had to undergo an enhanced chest CT scan and an abdominal CT scan at least every 2–3 months during the treatment period. These scans had to be an enhanced helical CT scan with 3–5 mm reconstruction. Tumor calcification and response evaluations were evaluated independently by two experienced radiologists. Tumor calcification was defined by the density in primary or metastatic lesions, with a CT value above 60 HU (Fig. 1). Metastatic lymph node calcification was defined as newly emerged calcification or enlarged calcification during treatment, excluding baseline calcification. During each evaluation, radiologists compared the current CT images with the previous CT images and measured the tumor calcification parameters (including the location and time of emergent calcification, number, density, and changes in calcification). Response evaluations were performed according to RECIST 1.1. Patients' clinical and pathological features, treatment options, other collected information, and survival follow-up findings were reviewed and collected by the oncologists from the Hospital Information Manage System, and final follow-ups were performed by telephone. OS was calculated from cetuximab treatment to death by any cause. PFS was defined as the period from the first day of cetuximab treatment to the time of tumor progression or death. The overall response rate (ORR) represents the total rate of complete responses and partial responses.

Analysis methods

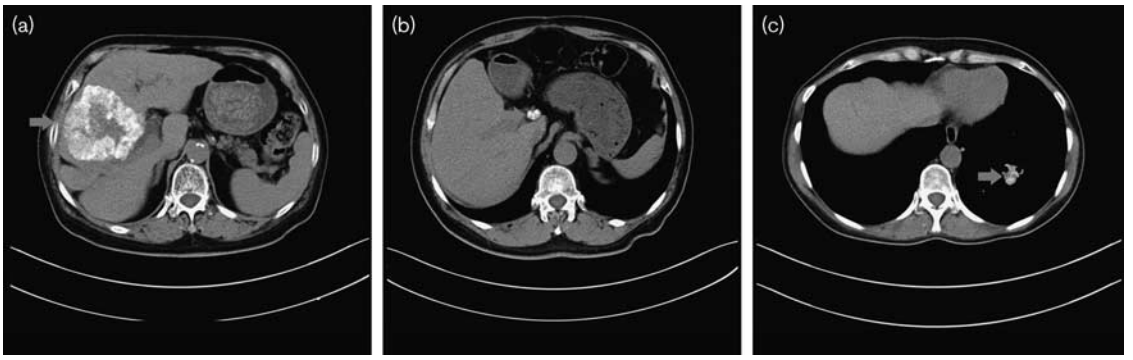
The statistical significance of differences between clinical baseline characteristics, including sex, age, blood test results, pathological features, and treatment strategies, was calculated using the χ^2-test or Fisher's exact test. The relationships among tumor calcification, nontumor calcification, and calcification characteristics (baseline calcification, calcification organ distribution, change in calcification, and number of calcifications), and OS and PFS were analyzed using the Kaplan–Meier method, and the P value was calculated using the log-rank test. The difference in the ORR between the tumor calcification group and the nontumor calcification group was analyzed using the χ^2-test. A P value less than 0.05 indicated statistical significance. SPSS 21.0 (SPSS Inc., Chicago, Illinois, USA) was used for all the data analyses.

Results

Clinical characteristics

We screened more than 300 patients in our hospital and only 111 patients (70 men and 41 women) with a mean age of 58 years who met the inclusion criteria. Twenty-seven [27/111 (24.3%)] patients had tumor calcification, among whom 13 had baseline calcification and 14 had post-therapy calcification. The median interval from treatment to the first appearance of tumor calcification was 2.0 months (95% confidence interval: 1.2–8.8 months). There were no statistically significant differences among patients with or without calcification in terms of sex, age, gene mutation, primary tumor location, pathological type, blood test result, or chemotherapy regimens. Notably, moderately differentiated carcinoma was more common in patients with calcification than in those without calcification, but this difference was not statistically significant (66.7 vs. 40.5%, $P = 0.074$; Table 1).

Fig. 1



The density of calcification was higher than adjacent soft-tissues whose computed tomography value was above 60 HU. The arrows point to the tumor metastatic calcification in (a) Liver, (b) lymph gland, and (c) lung.

Calcification features

Calcification features, including baseline status, organ distribution, changes during treatment, and number and location, were included in the analysis (Table 2). The number of calcifications increased during treatment in 25/27 (92.6%) patients with tumor calcification. Tumor calcification frequently occurred in the liver [21/27 (77.8%)] and lymph nodes [8/27 (29.6%)]. In terms of the calcification location by the trisection method, the central [22/27 (81.5%)] and marginal [20/27 (74.1%)] zones of tumor lesions were most common.

Correlation between calcification and survival or overall response rate

The last follow-up date was 1 March 2017 and the median follow-up time was 15.5 months. The median OS (mOS) and median PFS of the 111 patients were 15.5 and 7.7 months, respectively. Patients with tumor calcification had a significantly longer PFS than those without tumor calcification (9.3 vs. 6.2 months, $P=0.022$; Fig. 2). Patients with tumor calcification tended to have prolonged OS compared with patients without tumor calcification (21.9 vs. 16.5 months), but the difference was not statistically significant ($P=0.084$; Fig. 2). Furthermore, patients with tumor calcification had a significantly higher ORR than those without calcification (55.6 vs. 31.0%, $P=0.021$).

Correlation between calcification features and patient prognosis

Calcification features, including baseline status, organ distribution, changes during treatment, number, and location, were considered in the Kaplan–Meier analysis (Table 3). New calcification (22.9 vs. 15.9 months, $P=0.917$), liver metastasis calcification (21.9 vs. 18.7 months, $P=0.161$), and multiple tumor calcification (21.9 vs. 18.7 months, $P=0.651$) showed trends with better OS, but these correlations were not statistically significant. However, patients with an increasing number of calcifications after treatment had a significantly longer mOS (22.9 vs. 9.1 months, $P=0.033$). Only two patients with tumor calcification did not experience a change in the number of calcifications during treatment. Therefore, the 95% confidence interval could not be calculated, and the mOS was only 9.1 months. No calcification features were related to PFS (Table 3).

Discussion

In mCRC patients, liver metastasis, lymph node metastasis, and peritoneal metastasis are common and likely to calcify [15]. The relationship between tumor calcification and prognosis in patients with mCRC is still unclear, and few studies have focused on this relationship. Previous studies have suggested that liver tissue is prone to calcification [16], and post-treatment shrinkage, disappearance, or calcification of liver metastases in patients with mCRC were reported as signs of good prognosis [13]. In contrast to these studies, our study is the first to report the incidence, changes, and characteristics of tumor calcification, as well as the relationship between calcification and prognosis, in patients treated with cetuximab in combination with chemotherapy. In this study, the overall rate of calcification [27/111 (24.3%)] among patients who received cetuximab plus chemotherapy was higher than that among previously described patients who received chemotherapy alone [9,13]. In addition, 77.8% (21/27) of calcifications occurred in liver metastases, 25/27 (92.6%) patients with primary tumor calcification presented an increasing number of calcifications, and new calcifications occurred in 14/27 (51.9%) patients during treatment; these patients had a better mOS. The rate of post-therapy calcification was 4.0% in a previous study [14], but 12.6% in our study. The most common calcification location in our study was the central zone, which is in agreement with the findings reported by Hale *et al.* [14]. We hypothesized that the higher rate of calcification may be caused by cetuximab.

Tumor calcification is more likely to appear during treatment with cetuximab, but the physiopathological mechanism is unclear. The hypotheses are as follows: (i) Cetuximab can directly block epidermal growth factor receptor downstream

Table 1 The correlation between clinical characteristics and calcification

Characteristics	Total evaluated [*n* (%)] 111 (100)	Calcification [*n* (%)] Yes [27 (24.3)]	Calcification [*n* (%)] No [84 (75.7)]	*P* value
Sex				
Male	70 (63.1)	17 (63.0)	53 (63.1)	0.990[a]
Female	41 (36.9)	10 (37.0)	31 (36.9)	
Median age (range): 58.27 (27–79) (years)				
Age ≤ 70	94 (84.7)	24 (88.9)	70 (83.3)	0.696[a]
Age > 70	17 (15.3)	3 (11.1)	14 (16.7)	
Primary tumor site				
Rectum	47 (43.3)	12 (44.4)	35 (41.7)	0.868[a]
Left colon	35 (31.5)	9 (33.3)	26 (31.0)	
Right colon	29 (26.1)	6 (22.2)	23 (27.4)	
Number of metastases				
1	67 (60.3)	14 (51.9)	53 (63.1)	0.299[a]
≥ 2	44 (39.6)	13 (48.1)	31 (36.9)	
Tumor differentiation				
Poor	34 (30.6)	4 (14.8)	30 (35.7)	0.074[b]
Moderate	52 (46.8)	18 (66.7)	34 (40.5)	
Well	1 (0.9)	0 (0)	1 (1.2)	
Unknown	24 (21.6)	5 (18.5)	19 (22.6)	
BFAF mutation				
Wild type	60 (54.1)	17 (63.0)	43 (51.2)	0.528[b]
Mutant type	1 (0.9)	0 (0)	1 (1.2)	
Unknown	50 (45)	10 (37.0)	40 (47.6)	
Combined chemotherapy lines				
First line	77 (69.4)	21 (77.8)	59 (70.2)	0.447[b]
Second line	31 (27.9)	6 (22.2)	25 (29.8)	
Combined chemotherapy regimen				
FOLFOX	36 (32.4)	10 (37.0)	26 (31.0)	0.694[a]
FOLFIRI	69 (62.1)	15 (55.6)	54 (64.3)	
Irinotecan	6 (5.4)	2 (7.4)	4 (4.8)	
Alkaline phosphatase				
Normal (51–160 IU/l)	5 (4.5)	1 (3.7)	4 (4.8)	1.000[a]
> 160 IU/l	106 (95.5)	26 (96.3)	80 (95.2)	
< 51 IU/l	0 (0)	0 (0)	0 (0)	
Leukocyte				
Normal ($3.5–9.5 \times 10^9$/l)	98 (88.29)	22 (81.5)	76 (90.4)	0.351[a]
$> 9.5 \times 10^9$/l	7 (6.3)	2 (7.4)	5 (6.0)	
$< 3.5 \times 10^9$/l	6 (5.4)	3 (11.1)	3 (3.6)	
Lymphocyte				
Normal ($1.1–3.2 \times 10^9$/l)	83 (74.77)	20 (74.1)	63 (75.0)	0.884[b]
$> 3.2 \times 10^9$/l	2 (1.8)	0 (0)	2 (2.4)	
$< 1.1 \times 10^9$/l	26 (23.42)	7 (25.9)	19 (22.6)	
Creatinine				
Normal (53–140 μmol/l)	102 (91.9)	26 (96.3)	76 (90.5)	0.755[b]
> 140 μmol/l	1 (0.9)	0 (0)	1 (1.2)	
< 53 μmol/l	8 (7.2)	1 (3.7)	7 (8.3)	
Calcium				
Normal (2.1–2.7 mmol/l)	107 (96.4)	27 (100)	80 (95.2)	0.570[b]
> 2.7 mmol/l	0 (0)	0 (0)	0 (0)	
< 2.1 mmol/l	4 (3.6)	0 (0)	4 (4.8)	
Magnesium				
Normal (0.67–1.04 mmol/l)	105 (94.6)	24 (88.9)	81 (96.4)	0.309[a]
> 1.04 mmol/l	6 (5.4)	3 (11.1)	3 (3.6)	
< 0.67 mmol/l	0 (0)	0 (0)	0 (0)	
Phosphorus				
Normal (0.81–1.45 mmol/l)	105 (94.6)	27 (100)	78 (92.9)	0.494[b]
> 1.45 mmol/l	5 (4.5)	0 (0)	5 (6.0)	
< 0.81 mmol/l	1 (0.9)	0 (0)	1 (1.2)	

[a]χ^2-test.
[b]Fisher's exact test.

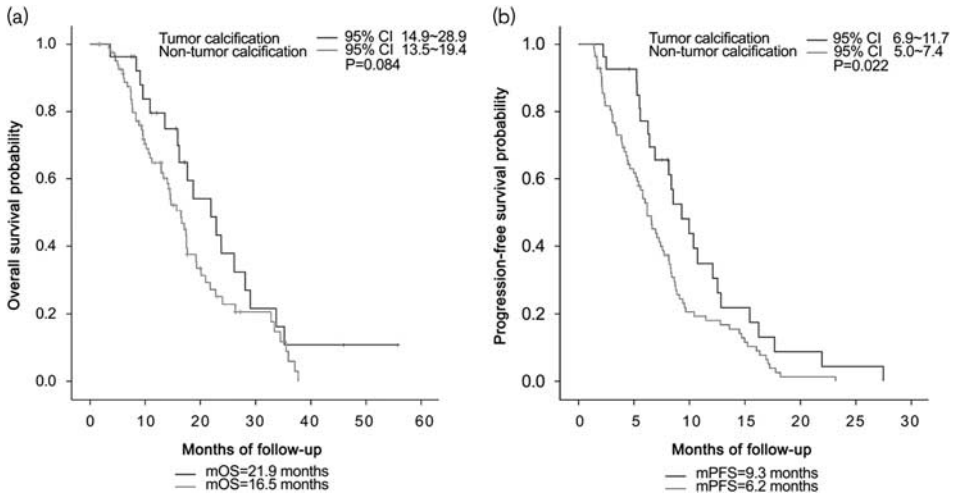
Table 2 The tumor calcification features of all patients ($n = 28$)

	n (%)
Baseline calcification	
Yes	13 (48.1)
No	14 (51.9)
Calcification organ distribution	
Liver	17 (63.0)
Lung	1 (3.7)
Lymph nodes	4 (14.8)
Primary lesion	1 (3.7)
Liver and lymph nodes	4 (14.8)
Increase of calcification	
Yes	25 (92.6)
No	2 (7.4)
Calcification number	
Single	14 (51.9)
Multiple	13 (48.1)
Calcification location	
Central	22 (81.5)
Region	17 (63.0)
Marginal	20 (74.1)

signaling pathways, resulting in tumor cell necrosis or apoptosis. The dead cells would be replaced rapidly with different kinds of inflammatory cells, leading to secondary chronic inflammatory reactions such as calcification. In contrast to cetuximab treatment, antiangiogenic therapy (e.g. bevacizumab) is characterized by intratumoral necrosis during treatment [17], and the lesion density on imaging decreases after treatment [18]. (ii) Cetuximab may cause different degrees of hypomagnesemia [19–21], leading to enzyme metabolic disorder; changes in cell membrane permeability; accelerated sodium-dependent, potassium-dependent, and calcium-dependent energy pump consumption; increased intracellular calcium storage; increased catecholamine and prostaglandin synthesis; and reduced blood flow. All these alterations can result in cell necrosis and tumor calcification. (iii) Tumor calcification might be dystrophic calcification secondary to necrosis and hemorrhage before or after cetuximab treatment [22].

In this study, patients with tumor calcification who had received cetuximab combined with chemotherapy showed significantly improved PFS, OS, and ORR than those without tumor calcification. However, the results of previous studies on tumor calcification and prognosis are contradictory. Easson *et al.* [13] noted that CRC calcification was an important prognostic marker of survival benefit, but was not associated with tumor differentiation, tumor type, or hepatic tumor burden. The chemotherapy regimens used in the study by Easson included 5-FU, folinic acid, and *N*-phosphonacetyl-L-aspartic acid. In contrast, Hale *et al.* [14] analyzed the correlations among calcification percentage, properties, and location and treatment efficacy and suggested that calcification was not related to the prognosis of patients with colorectal carcinoma. In this previous study, patients were treated with 5-FU-based chemotherapy. The survival of patients with tumor calcification was 11 months before chemotherapy and only 9.17 months after treatment. These previous studies were carried out before the targeted treatment era. Apart from the use of 5-FU-based chemotherapy, the addition of cetuximab to the therapeutic regimen might have been the main cause of the discrepancies between our results and those of the previous two studies. On the

Fig. 2



Kaplan–Meier estimates of overall survival (OS) and progression-free survival (PFS) in metastastic colorectal cancer (mCRC) patients with and without tumor calcification. (a) OS among the 111 patients. Median overall survival (mOS) time in the tumor calcification was 21.9 months [(95% confidence interval (CI): 14.9– 28.9)], compared with 16.5 months (95% CI: 13.5–19.4) in the nontumor calcification group. The *P* value was 0.084 by log-rank test. (b) PFS among the 111 patients. Median progression-free survival (mPFS) time in the tumor calcification was 9.3 months (95% CI: 6.9–11.7), compared with 6.2 months (95% CI: 5.0–7.4) in the nontumor calcification group. The *P* value was 0.022 by log-rank test.

Table 3 The correlation between calcification features and patients prognosis

Variables	mOS (months)	95% CI	*P* value	mPFS (months)	95% CI	*P* value
Baseline calcification						
Yes	15.9 ± 3.8	8.4–23.4	0.917	10.4 ± 3.5	3.4–17.3	0.545
No	22.9 ± 1.5	20.0–26.0		9.3 ± 1.3	6.7–12.0	
Calcification organ distribution						
Liver	21.9 ± 7.0	8.2–35.6	0.161	8.5 ± 0.77	7.0–10.0	0.05
Other sites	18.7 ± 4.3	10.3–27.0		9.97 ± 3.9	2.2–17.7	
Increase number of calcification						
Yes	22.9 ± 3.43	16.1–29.6	0.033	9.3 ± 1.1	7.1–11.5	0.162
No	9.1	–		5.5	–	
Calcification number						
Single	18.7 ± 5.1	8.6–28.8	0.651	8.5 ± 1.7	5.2–11.9	0.810
Multiple	21.9 ± 7.4	7.3–36.5		9.3 ± 1.3	6.7–11.9	

P value: log-rank.
CI, confidence interval; mOS, median overall survival; mPFS, median progression-free survival.

one hand, we found that patients with an increasing number of calcifications after treatment, new calcifications, liver metastasis calcification, and multiple tumor calcification had a trend toward better OS. On the other, we also focused on the relationship between tumor calcification and the baseline inflammatory cell ratio and electrolyte level, but found no statistically significant differences. More patients will hopefully be included in this type of study in the future.

There are some limitations to our study. This study was a retrospective single-center study with a limited number of cases. We had to enroll all patients who had received first-line or second-line cetuximab treatment, which may have led to the shorter OS in our report than that in the literature on first-line cetuximab therapy. Moreover, the chemotherapy protocols in this study were not uniform.

Conclusion

Tumor calcification predicts a survival benefit and a better response rate in mCRC patients treated with cetuximab and chemotherapy. Tumor calcification and an increasing number of calcifications are positive prognostic factors for survival. All these discoveries are unprecedented and provide a solid foundation for further study on the correlation between tumor calcification and prognosis in oncology.

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Conflicts of interest

There are no conflicts of interest.

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