Clinicopathological and mutational differences between tumors with multiple metastases and single lung metastasis in colorectal cancer

YUKA YANAI1, TAKUO HAYASHI1, YOICHI AKAZAWA2, NOBORU YATAGAI2, SHO TSUYAMA1, TAKASHI YAO1 and TSUYOSHI SAITO1,3

Departments of 1Human Pathology and 2Gastroenterology; 3Intractable Disease Research Center, Juntendo University, Graduate School of Medicine, Tokyo 113-8421, Japan

Received June 26, 2019; Accepted March 2, 2020

DOI: 10.3892/ol.2020.11627

Abstract. Cancer metastasis, particularly multiple metastatic cancer, is a significant event that affects patient prognosis. However, single metastasis can be treated by partial resection, although the clinicopathological and molecular profile of single lung metastasis has not been thoroughly elucidated. The present study examined tumor heterogeneity by comparing the mutation status between primary colorectal cancer (CRC) and corresponding metastatic lesions to identify prognostic factors associated with single lung metastasis and multiple metastases. The present study enrolled 31 cases of CRC, 20 cases with multiple metastases and 11 cases with single lung metastasis. Clinicopathologically, all cases with multiple metastases were tubular adenocarcinoma, and 3/11 cases with single metastasis were mucinous adenocarcinoma originating from the left side, the remaining 8 cases were tubular adenocarcinoma from the left side. CRC cases with multiple metastases exhibited more frequent vascular invasion, but not lymphatic invasion, than those with single lung metastasis. Furthermore, CRC with multiple metastases was associated with strong tumor budding (P=0.04). Patients with CRC with multiple metastases had lower recurrence-free survival rates compared with those with single lung metastasis (P=0.02). However, there was no significant difference between these two groups in terms of overall survival rates. A next-generation sequencing cancer hotspot panel was used to analyze a heterochronous multiple metastases case, including brain metastasis. Sanger sequencing, immunohistochemistry and microsatellite instability were examined for all 31 cases to reveal the molecular features. KRAS and TP53 mutation signatures were largely preserved throughout the metastatic events. TP53/APC mutations and overexpression of p53 appeared to be associated with the presence of lymphovascular invasion and strong tumor budding, respectively, although these differences were not statistically significant. Early relapses in patients with CRC could be a sign for eventual multiple metastases, although these may not affect the overall survival of patients with CRC. Considerable mutational changes were seemingly rare during metastatic events in patients with CRC.

Introduction

Metastasis is an important event that defines patients’ prognosis during cancer treatment. Cancer cells acquire a variety of phenotypes that allow them to adapt to the distinct tissue microenvironment during the metastatic process. Mutational and epigenetic changes in cancer cells strongly characterize these events. In colorectal cancer (CRC), KRAS, NRAS, BRAF, and PIK3CA mutations occur as oncogenic drivers, which are also expected to play an essential role in these metastatic events.

The liver is the most frequently invaded metastatic organ in CRC, and several studies have demonstrated mutational signatures associated with CRC liver metastasis (1). CRC liver metastasis, even in multiple metastatic cases, can be potentially cured by hepatic resection. Lung metastasis occasionally occurs without liver metastasis; however, mutational signatures associated with single lung metastasis are not well known. Single lung metastasis is also curable by partial resection of the lung. A better understanding of the metastatic potential of primary CRCs would be significantly beneficial in the treatment of CRC patients.
The mutational signature differences between primary and metastatic lesions, especially those associated with single lung metastasis or multiple metastases, has remained unclear. A comparison of the clinicopathological and mutational profiles associated with multiple/single lung metastases in CRC could unravel the fundamental mechanisms underlying tumor metastasis and help to identify early detection biomarkers.

The current study aimed to investigate the clinicopathological and molecular features of tumor heterogeneity by comparing the mutation status between the primary tumor and corresponding metastatic lesions in order to detect factors associated with multiple tumor metastases (which are usually associated with worse prognosis).

Materials and methods

Case selection and histological evaluation. A total of 2,912 cases of CRC were surgically resected at the Juntendo University Hospital (Tokyo, Japan) between 2003 and 2017. We collected data and tissues from 31 CRC cases with lung metastasis (20 with multiple metastases and 11 with single metastasis) from the pathological record. The following clinicopathological factors were evaluated: Gender, age, tumor location, tumor size, histological type, lymphovascular invasion, tumor budding, poor differentiated cluster, perineural invasion, cancer stroma, depth of invasion, lymph node metastasis, distant metastasis, and tumor-node-metastasis (TNM) stage. TNM staging was determined using the 8th UICC TNM staging system of tumors of the colon and rectum (2). The presence of tumor budding (TB) and poorly differentiated cluster (PDC) were evaluated at the invasion front as previously described. TB was counted in the area with the highest density and classified as follows: BD1: 0-4; BD2: 5-9; and BD3: ≥10 (x200 magnification). Furthermore, PDC was classified into three groups: G1, G2, and G3, when they have a maximum number of ≤5, 5-9, ≥10 PDC, respectively; the counting was done in the highest density area at x200 magnification (3). All patients were followed-up every three months after surgery. The survival periods were determined as survival times after diagnosis. The mean follow-up time was 69.7 months (the range was 18-178 months).

Next-generation sequencing (NGS). A CRC sample with heterochronous multiple metastases, including brain metastasis, was subjected to NGS using the Ion Ampliseq Cancer Hotspot Panel v2 (Thermo Fisher Scientific, Inc.). The sequences were then examined by using a capillary sequencing machine (3730xl Genetic Analyzer; Applied Biosystems; Thermo Fisher Scientific, Inc.) and analyzed with the capillary sequencing software (version 3.5.1 software (Applied Biosystems; Thermo Fisher Scientific, Inc.). The sequences were then examined by using Sequencing Analysis software version 5.0.1 software (Applied Biosystems; Thermo Fisher Scientific, Inc.). Mutations were evaluated by two of the authors (Y.Y. and T.S.) and registered if the mutation peak height reached 20% of the normal peak height. All mutations were verified by sequencing the sense and antisense strands.

Microsatellite instability. Microsatellite instability (MSI) analysis was performed using five markers (Bethesda panel: BAT25, BAT26, D5S346, D2S123, and D17S250) (5). Samples with two or more altered markers were classified as MSI-high (MSI-H), samples with one altered marker were classified as MSI-low (MSI-L), and samples without altered markers were classified as microsatellite stable (MSS). We used the primer sets for highly fragmented DNA extracted from the FFPE tissue (6).

Survival analysis and statistical analysis. Correlations between clinicopathological factors and genetic alterations were analyzed by the Fisher’s exact test, chi-squared test, and Student’s t-test. To elucidate the prognostic impact of each factor, we performed Kaplan-Meier survival analysis and log-rank tests. P<0.05 was considered to indicate a
Clinicopathological differences between CRC with multiple metastases and single metastasis. Clinicopathological differences between CRC with multiple metastases and that with single lung metastasis are summarized in Table I. In this study, the incidence of the single lung metastasis was 0.38% and the cases in which the first metastatic focus was observed in the lung was 0.52% among the multiple metastases group (multiple metastases: 4, single lung metastasis: 11). All the cases with multiple metastases were tubular adenocarcinoma, whereas 3 of 11 cases with single metastasis were mucinous adenocarcinoma. CRCs, which eventually caused multiple metastases, were located evenly from the right of the rectal origin of primary tumors, whereas none of the CRCs with single lung metastasis arose from the right-sided colon, although this difference was not statistically significant (P=0.06). CRC with multiple metastases more frequently showed vascular invasion, but not lymphatic invasion, than those with single lung metastasis. Furthermore, CRC with multiple metastases was associated with strong tumor budding (P=0.04). The presence of PDC did not affect single or multiple metastatic states. CRC patients with multiple metastases presented shorter recurrence-free survival rates compared with those with single lung metastasis with statistical significance (P=0.02). However, there was no significant difference between these two groups regarding overall survival rates. The impact of KRAS, TP53, and APC mutation signatures and IHC of p53 overexpression status on clinicopathological factors were also assessed. TP53 and APC mutations seemed to be associated with the presence of lymphovascular invasion and strong tumor budding, respectively, although these differences were not statistically significant (Tables II and III).
The overexpression of p53 tended to be associated with vascular invasion, but this association was not statistically significant (Table IV). No significant association was found with KRAS mutation (Table SII).
KRAS mutation signatures according to metastatic events (Fig. 1). KRAS mutations were found in 12 out of 20 cases with multiple metastases at the primary sites, and in 7 out of 11 cases with single metastasis at the primary sites. KRAS mutation signatures were maintained throughout the metastatic events in most cases. In two cases (Case #M19 and #S2), clones different from the primary sites were detected at the metastatic sites, and in one case (Case #M20), clones with KRAS mutation were first detected at the metastatic site, whereas no KRAS mutation was detected in the primary tumor. Both metastatic brain lesions in Case #M5 harbored KRAS mutations similar to that of the primary tumor, and only two out of the other seven metastatic lesions contained KRAS mutations.

TP53 mutation signatures and p53 immunohistochemistry, and the relationship with metastatic events (Fig. 1). TP53 mutations were found at the primary sites in 9 of 20 cases with multiple metastases, and in 3 of 11 cases with single metastasis at the primary sites. In one patient (Case #M14), a TP53 mutation was detected in the latest metastatic lesion, despite the absence of a TP53 mutation at the primary site. In another patient (Case #M20), clones with KRAS mutation were first detected at the metastatic site, whereas no KRAS mutation was detected in the primary tumor. Both metastatic brain lesions in Case #M5 harbored KRAS mutations similar to that of the primary tumor, and only two out of the other seven metastatic lesions contained KRAS mutations.

APC mutation signatures according to the metastatic events (Fig. 1). APC mutations were detected in 8 out of 20 cases with multiple metastases at the primary sites, and in 3 out of 11 cases with single metastasis at the primary sites. All mutations were considered frameshift or nonsense, and the mutation signatures were preserved throughout the multiple metastatic events. Regarding a patient with metastatic brain lesions (Case #M5), five out of seven metastatic lesions contained TP53 mutations, in addition to the brain metastatic tumors. The overexpression of p53 was observed in 11 of 20 multiple metastatic cases at the primary site (55.0%), 9 of which had a TP53 mutation. Eight of 9 cases with a mutation in the primary site showed overexpression of p53 in all the metastatic sites; however, TP53 mutations were not preserved in all of the metastatic lesions (Cases #M1, #M5, and #M7). In Case #M5, the overexpression of p53 was not observed in two metastatic lesions of the liver, one of which contained a TP53 mutation. In Case #M14, p53 overexpression was detected only in metastatic lesions. A TP53 mutation was absent in the primary site and detected only in the second metastatic site in the lung. In patients with single metastasis, the overexpression of p53 was observed in 4 of 11 cases (36.3%), 3 of which had a TP53 mutation in the primary tumors. In one of the three cases with single lung metastasis, the overexpression of p53 was also observed at the metastatic site without a mutation (Case #S7). We also observed the overexpression of p53 both at the primary and metastatic sites in three cases without a TP53 mutation (Case #M6, #M11, and #S3). There were no statistically significant differences between multiple metastases and single lung metastasis in terms of mutation ratio and overexpression of p53 (P=0.45 and P=0.46, respectively).

Table IV. Association between p53 immunohistochemistry and clinicopathological factors.

| Variable               | p53 overexpression, n | P-value | \(\chi^2\) | Fisher |
|------------------------|-----------------------|---------|------------|--------|
|                        | Positive | Negative |           |        |
| Poorly differentiated clusters | 4/8/3 | 2/12/2 | 0.44 | 0.51 |
| Budding grade G1/2/3 | 12/0/3 | 12/1/3 | 0.51 | >0.99 |
| PN No/yes             | 12/3 | 14/2 | 0.65 |        |
| Ly No/yes             | 7/8 | 8/8 | >0.99 |        |
| V No/yes              | 3/12 | 7/9 | 0.25 |        |
| Pathologic type       | 13/2 | 15/1 | 0.60 |        |
| Location              | 3/5/7 | 2/4/10 | 0.67 | 0.69 |
| pStage I/II/IIIA/IIIB/IV | 1/1/6/1/6 | 2/1/5/2/6 | 0.51 | >0.99 |

PN, perineural invasion; Ly, lymphatic invasion; V, vascular invasion; tub, tubular adenocarcinoma; muc, mucinous adenocarcinoma.
Clinicopathological and molecular differences between CRC with multiple lung metastases and that with single lung metastasis. There were two cases of multiple lung metastases without metastasis to other organs (Case #M10 and #M16). The primary tumors were located in the rectum in both cases, and both tumors harbored a KRAS mutation. Furthermore, these two cases tended to show histologically higher PDC grade, BD grade, and vascular invasion compared with those with single lung metastasis. It seemed that the primary tumors that can eventually cause multiple lung metastases have higher metastatic potential compared with single lung metastatic cases. However, there were no significant differences between any clinicopathological and molecular characteristics of multiple lung metastases and those of single lung metastasis, although the study was limited by the small sample size (Table SIII).

Sanger sequencing for other genes. Sanger sequencing was performed for TERT, CTNNB1, BRAF, CRAF, NRAS, PIK3CA, and GNAS. No hot spot mutations were detected in these genes.

Wnt signal activation in metastatic CRC. Due to the frequency of APC (40.0% in multiple metastatic cases, 27.3% in single lung metastatic cases) and CTNNB1 (0%) mutations, the mutations in these series of cases seemed to be relatively rare.
compared with reported values (7‑9). The status of Wnt signal activation was assessed by β‑catenin nuclear staining. Four out of 20 cases of multiple metastases did not show β‑catenin nuclear staining, whereas one of them harbored an APC mutation. The average β‑catenin nuclear labeling index was 34.3% in multiple metastatic cases and 40.7% in single lung metastatic cases (Table SIV).

Status of microsatellite instability in metastatic CRC. The status of MSI was also assessed for the primary tumors. MSI‑L was found in only two of the multiple metastatic cases (Case #M7 and #M16) and the remaining tumors were classified as MSS (Fig. 2).

Prognostic impacts of clinicopathological factors and metastatic state. TNM stage at the time of the primary surgery significantly affected the patients' overall survival rate (OSR) and time to the first recurrence (TFR). TFR was significantly shorter for the patients who eventually experienced multiple metastases than for patients with single metastasis (Fig. 3A; P=0.02). However, although OSR was slightly worse for patients who experienced eventual multiple metastases, this finding was not statistically significant (Fig. 3B; P=0.77).

Discussion

It has been reported that lung metastasis occurs in 0.67‑15.8% of CRC (10,11). In this study, the incidence of single lung metastasis was 0.38%, and the cases in which the first metastatic focus was observed in the lung was 0.52% of the multiple metastases group. The incidence rate in our study seems to be lower than previously reported values. However, the previous studies probably included ‘suspected cases’ examined only by computed tomography (CT) or plain radiography, and the present study only analyzed cases that were surgically resected and histologically proven as metastasis. These differences might have influenced the difference in the incidence. However, our findings suggest that the first metastatic event can be observed in the lung in approximately 0.9% of CRC cases.

In this study, all CRC cases of right‑sided origin belonged to the multiple metastatic group, which is consistent with the
finding that right-sided primary CRCs show worse prognosis than left-sided primary tumors (7,8,12). In contrast, it was unexpected that the single lung metastatic group contained three cases of mucinous carcinoma, and none were found in the multiple metastatic group. Mucinous carcinoma, except for those with MSI-H, tends to show worse prognosis than conventional adenocarcinoma (13-16). We examined MSI status in the cases, but MSI-H was not found in any of the cases. Therefore, the reason for the single metastasis of the aggressive mucinous carcinoma to the lung, but not to other organs, is unclear.

We included PDC and TB as pathological factors and evaluated if these factors are associated with metastatic status, as a recent study demonstrated that a grading system using PDC and TB in neoplastic cells is a strong predictor of nodal metastases and adverse outcome in colon cancer (17). Strong TB was frequently observed in the group with multiple metastases and adverse outcome in colon cancer (17). Strong TB was frequently observed in the group with multiple metastases and adverse outcome in colon cancer (17). Strong TB was frequently observed in the group with multiple metastases and adverse outcome in colon cancer (17). Strong TB was frequently observed in the group with multiple metastases and adverse outcome in colon cancer (17). Strong TB was frequently observed in the group with multiple metastases and adverse outcome in colon cancer (17). Strong TB was frequently observed in the group with multiple metastases and adverse outcome in colon cancer (17). Strong TB was frequently observed in the group with multiple metastases and adverse outcome in colon cancer (17). Strong TB was frequently observed in the group with multiple metastases and adverse outcome in colon cancer (17). Strong TB was frequently observed in the group with multiple metastases and adverse outcome in colon cancer (17). Strong TB was frequently observed in the group with multiple metastases and adverse outcome in colon cancer (17). Strong TB was frequently observed in the group with multiple metastases and adverse outcome in colon cancer (17). Strong TB was frequently observed in the group with multiple metastases and adverse outcome in colon cancer (17).

In this study, both lesions of brain metastasis contained KRAS mutations similar to that detected in the primary tumor, whereas only two out of seven metastatic tumors from the same patient contained this type of mutation. Our findings are consistent with previous findings and also provide evidence that anti-epidermal growth factor receptor therapy is not effective for the treatment of metastatic brain tumors in CRC.

Regarding liver metastasis, 12 out of 20 cases in multiple metastatic tumors harbored KRAS mutations in the primary tumor, and 8 of these 12 patients developed liver metastasis. Seven out of 14 metastatic liver samples from the eight patients did not harbor KRAS mutations. Liver metastasis is associated with TOP2A gene amplification but not with a high frequency of KRAS mutation, although TOP2A gene amplification was not examined in our series (24). A previous study reported that tumors with KRAS mutations were more likely to develop lung metastasis. However, the overall survival did not differ according to the KRAS status (25). In this study, 12 out of 20 (60.0%) cases with multiple metastases and 7 out of 11 (63.6%) cases with single lung metastasis harbored a KRAS mutation at the primary sites.

Furthermore, there was no significant difference between the two groups with regards to overall survival. Thus, KRAS mutation status did not affect single/multiple lung metastases in this study. Moreover, in this study, BRAF mutations were not detected in many cases, which is consistent with previous findings (24).

With respect to treatment, the general condition of the patients allowed the resection of the metastatic lesions on several occasions in most of the patients with multiple metastases. It is reported that the resection of liver and lung metastases provides good long-term survival (26). All patients who succumbed from the disease (multiple metastasis: 3, single lung metastasis: 1) could not continue with the postoperative adjuvant chemotherapy or undergo subsequent surgery due to poor performance status and the side effects of the drugs.

Finally, CRC is known, in general, to initially spread to the liver, and then to the lung and the brain. It was reported that synchronous liver and pulmonary metastases occur in 45 to 70% of patients with CRC (9). Besides, lung metastasectomy in patients with previously resected liver metastases showed a significantly better five-year survival (27). Closer observation of the liver and lung metastases is needed to improve the prognosis of patients. The rationales for comparing single lung metastasis and multiple lung metastases in CRC in the current study are as
follows: (1) clinicians need to carefully follow-up the patients who experienced early relapse, as they have a higher risk of multiple metastasis in the near future. (2) Lung metastasis from CRC is usually encountered after liver metastasis. In the case of single lung metastasis after CRC, the possibility of primary pulmonary adenocarcinoma with enteric differentiation needs to be ruled out (28,29). Frequently conserved mutations in TP53, APC, and KRAS, together with p53 IHC findings, could help to distinguish metastasis from primary pulmonary adenocarcinoma with enteric differentiation.

In conclusion, early relapses in CRC patients could be a sign of eventual multiple metastases, although this may not affect the overall survival of CRC patients. Drastic mutational changes seem rare during metastatic events in CRC patients.

A few limitations can be considered in this study. First, the numbers of the cases were too small to draw definitive conclusions. The sample number should be the same in each group. However, based on available pathological records, we found only 31 CRC cases with lung metastasis (20 with multiple metastases and 11 with single metastasis) from amongst 2,912 cases of CRC. Therefore, it is not possible to increase the number of cases. More sample accumulation is necessary to find more persuasive correlations or differences. Second, we verified mutation findings only in TP53 but not in APC and KRAS. We employed p53 IHC to verify the mutation findings, because it is well known that p53 IHC antibody is able to detect mutated p53 as overexpression. However, there are no commercially available IHC antibodies that can efficiently detect mutated APC and KRAS, making it difficult to confirm the mutation findings.

Acknowledgements

Not applicable.

Funding

The present study was financially supported by a Grant-in-Aid for General Scientific Research from the Ministry of Education, Science, Sports and Culture (grant no. 17K08704 to TY; Tokyo, Japan).

Availability of data and materials

All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

TS, TH and TY planned this study and diagnosed the surgical specimens. YY, YA, NY and ST performed the experiments and analyzed the data. YY and TS wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Juntendo University, Tokyo, Japan (approval no. 17-214), and all patients provided written informed consent prior to enrollment.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Taki K, Ohmuraya M, Tanji E, Komatsu H, Hashimoto D, Semb K, Araki K, Kawaguchi Y, Baba H and Furukawa T: GNAS(R201H) and Kras(G12D) cooperate to promote murine pancreatic tumorigenesis recapitulating human intraductal papillary mucinous neoplasm. Oncogene 35: 2407-2412, 2016.
2. Brierley JD, Gospodarowicz MK and Wittekind C (eds): TNM classification of malignant tumors, 8th edition. Wiley-Blackwell, 2017.
3. Lee VWK and Chan KF: Tumor budding and poorly-differentiated cluster in prognostication in stage II colon cancer. Pathol Res Pract 214: 402-407, 2018.
4. Akazawa Y, Saito T, Hayashi T, Yanai Y, Tsuyama S, Akaike K, Suehara Y, Takahashi F, Takamochi K, Ueyama H, et al: Next-generation sequencing analysis for gastric adenocarcinoma with enteroblastic differentiation: Emphasis on the relationship with hepatoid adenocarcinoma. Hum Pathol 78: 79-88, 2018.
5. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN and Szrivastava S: A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition: Development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 58: 5248-5257, 1998.
6. Umetani N, Sasaki S, Watanabe T, Ishigami H, Ueda E and Nagawa H: Diagnostic primer sets for microsatellite instability optimized for a minimal amount of damaged DNA from colorectal tissue samples. Ann Surg Oncol 7: 276-280, 2000.
7. Cancer Genome Atlas Network: Comprehensive molecular characterization of human colon and rectal cancer. Nature 487: 330-337, 2012.
8. Jass JR, Young J and Leggett BA: Evolution of colorectal cancer: Change of pace and change of direction. J Gastroenterol Hepatol 17: 17-26, 2002.
9. Miyaki M, Iijima T, Kimura J, Yasuno M, Mori T, Hayashi Y, Koike M, Shitara N, Iwama T and Kuroki T: Frequent mutation of beta-catatin and APC genes in primary colorectal tumors from patients with hereditary nonpolyposis colorectal cancer. Cancer Res 59: 4506-4509, 1999.
10. Parnaby CN, Bailey W, Balasingam A, Beckert L, EGLINTON T, Fife J, FRIZZLE FA, Jeffery M and Watson AJ: Pulmonary staging in colorectal cancer: A review. Colorectal Dis 14: 660-670, 2012.
11. Mitry E, Guin B, Cosconea S, Jooste V, Faivre J and Bouvier AM: Epidemiology, management and prognosis of colorectal cancer with lung metastases: A 30-year population-based study. Gut 59: 1383-1388, 2010.
12. Boeckx N, Koukakis R, Op de Beeck K, Rolfo C, Van Camp G, Stien S, Tabernero J, Douillard JY, ANDRÉ T and Peeters M: Primary tumor sidedness has an impact on prognosis and treatment outcome in metastatic colorectal cancer: Results from two randomized first-line panitumumab studies. Ann Oncol 28: 1862-1867, 2017.
13. Kanemitsu Y, Kato T, Hirai T, Yasui K, Morimoto T, Shimizu Y, Kodera Y and Yamamura Y: Survival after curative resection for mucinous adenocarcinoma of the colorectum. Dis Colon Rectum 46: 160-167, 2003.
14. Jimi S, Hotokezaka M, Ikeda T, Uchiyama S, Hidaka H, Machida N, Ishizaki H and Chihiwa K: Clinicopathological characteristics and prognostic factors of advanced colorectal mucinous adenocarcinoma. Histopathology 61: 162-169, 2012.
16. Ott C, Gerken M, Hirsch D, Fest P, Fichtner-Feigl S, Munker S, Schnoy E, Stroszczynski C, Vogelhuber M, Herr W, et al.: Advanced mucinous colorectal cancer: Epidemiology, prognosis and efficacy of chemotherapeutic treatment. Digestion 98: 143-152, 2018.

17. Reggiani Bonetti L, Barresi V, Bettelli S, Caprera C, Manfredini S and Maiorana A: Analysis of KRAS, NRAS, PIK3CA, and BRAF mutational profile in poorly differentiated clusters of KRAS-mutated colon cancer. Human Pathol 62: 91-98, 2017.

18. Yaeger R, Chatila WK, Lipsyc MD, Hechtman JF, Cercek A, Sanchez-Vega F, Jayakumaran G, Middha S, Zehir A, Donoghue MTA, et al.: Clinical sequencing defines the genomic landscape of metastatic colorectal cancer. Cancer Cell 33: 125-136.e123, 2018.

19. Greaves M and Maley CC: Clonal evolution in cancer. Nature 481: 306-313, 2012.

20. Maley CC, Aktipsis A, Graham TA, Sottoriva A, Boddy AM, Janiszewska M, Silva AS, Gerlinger M, Yuan Y, Pienta KJ, et al.: Classifying the evolutionary and ecological features of neoplasms. Nat Rev Cancer 17: 605-619, 2017.

21. Siegmund KD, Marjoram P, Woo YJ, Tavaré S and Shibata D: Inferring clonal expansion and cancer stem cell dynamics from DNA methylation patterns in colorectal cancers. Proc Nat Acad Sci USA 106: 4828-4833, 2009.

22. Schell MJ, Yang M, Teer JK, Lo FY, Madan A, Coppola D, Monteiro AN, Nebozhyn MV, Yue B, Loboda A, et al.: A multi-gene mutation classification of 468 colorectal cancers reveals a prognostic role for APC. Nat Comm 7: 11743, 2016.

23. El-Deiry WS, Vijayvergia N, de Araujo LC, Rao N, Le M and Suster S: Cribriform adenocarcinoma of the lung: Clinicopathologic, immunohistochemical, and molecular analysis of 15 cases of a distinctive morphologic subtype of lung adenocarcinoma. Mod Pathol 27: 1063-1072, 2014.

24. Matsushima J, Yazawa T, Suzuki M, Takahashi Y, Ota S, Nakajima T, Yoshino I, Yokose T, Inoue T, Kawahara K and Nakatani Y: Clinicopathological, immunohistochemical, and mutational analyses of pulmonary enteric adenocarcinoma: Usefulness of SATB2 and β-catenin immunostaining for differentiation from metastatic colorectal carcinoma. Hum Pathol 64: 179-185, 2017.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.