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Unexpected tumor reduction in metastatic colorectal cancer patients during SARS-Cov-2 infection

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***Abstract*:** Herein, we describe three patients affected by metastatic colorectal cancer (mCRC) experiencing infection by severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) and reduction of disease burden during coronavirus disease 2019 (COVID-19) course. Insights into tumor-associated angiotensin-converting enzyme (ACE)-2 expression and lymphocyte function suggest a correlation between host/SARS-Cov-2 infection and tumor burden reduction. This may shed new light into (a) the infection mechanism of SARS-CoV-2 virus and (b) the multiple aspects of a composite antiviral immune response with potential paradoxical and unexpected applications.

Keywords: chemotherapy, colorectal cancer, COVID-19, NK cells, SARS-CoV-2

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Introduction

Colorectal cancer (CRC) is the third cause of cancer-related deaths worldwide. The therapeutic panorama has been enriched by biologic therapies [anti-angiogenic anti-EGFR (epidermal growth factor receptor) drugs] in association with standard chemotherapies (fluorouracil, irinotecan and oxaliplatin). However, the survival of metastatic disease rarely lasts 24 months.[1]

In the last year, since SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) has spread from Wuhan (China) to worldwide, many oncological patients undergoing COVID-19 (coronavirus disease 2019) experienced, among other effects, treatment delay. In fact, according to several institutional policies, patients positive to molecular pharyngo-nasal swabs cannot undergo restored, or commence, chemotherapy until after three consecutive negative molecular pharyngo-nasal swabs.

In this scenario, three patients affected by metastatic CRC (mCRC) displayed radiologic reduction of disease burden during their COVID-19 experience. The patients' consent for anonymized publication was obtained and archived in accordance with regulatory authorities.

Case descriptions

Patient 1 is a 65-year-old man who received a right hemicolectomy in October 2019. Histologic and genetic examination revealed a pT4apN1b *RAS* and *BRAF* wild-type, MSS (microsatellite stable) adenocarcinoma and he presented with liver metastases. Disease stabilization (with no change in tumor size) was obtained after 7 months of first-line chemotherapy FOLFOX/BEVA [oxaliplatin on day 1 at the dose of 85 mg/m^2 as a 2 h infusion, concurrently with leucovorin 400 mg/m^2, followed by a bolus of 5-fluorouracil 400 mg/m^2 (FU), and a 48 h infusion of 5-fluorouracil 2400 mg/m^2 (FA) and bevacizumab 5 mg/kg (BEVA) on day 1, every 2 weeks] as documented by CT (computed tomography) in August 2020. Following therapy was FU/FA/BEVA every 2 weeks as maintenance/depotentiated treatment, with the last cycle performed on 2 October 2020, for a total of three cycles. Thereafter, he developed

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severe COVID-19, from which he recovered in November 2020 when the CT scan showed complete response with regression of hepatic lesions (Figure 1). The immunoglobulin G (IgG) anti-SARS-CoV-2 titer was 1455 U/ml.

Patient 2 is a 58-year-old man who underwent a left hemicolectomy in February 2019. The histologic and genetic assessment showed a pT3pN0 *RAS* mutated, *BRAF* wild-type, MSS adenocarcinoma. At diagnosis, he presented with three unresectable liver metastatic lesions treated with pre-operative FOLFOX/BEVA (6 months) followed by multiple metastasectomies in November 2019. Thereafter, the patient refused post-operative chemotherapy. In August 2020, he progressed at liver segments VI and VII as documented with CT scan; concomitantly, he developed mild symptomatic COVID-19. CT scan in December 2020 reported reduction of the liver metastatic lesion at segment VII (15.3 × 14.0 mm *versus* 10.9 × 10.5 mm) and disappearance of the lesion at segment VI (Figure 1). The IgG anti-SARS-CoV-2 titer was 188.40 U/ml.

Patient 3 is a 60-year-old woman who received a left hemicolectomy in March 2019 for a pT3pN2a *RAS* and *BRAF* wild-type, MSS adenocarcinoma followed by 6 months adjuvant FOLFOX therapy (oxaliplatin on day 1 at the dose of 85 mg/m^2 as a 2 h infusion, concurrently with leucovorin 400 mg/m^2, followed by a bolus of FU and a 48 h infusion of FA). In June 2020, the disease progressed on positron emission tomography and CT total-body scan, with multiple measurable nodules on the peritoneum surface (standard uptake value > 3) and a small nodule in the right lung. The patient started first-line chemotherapy with FOLFIRI/PANI (irinotecan 180 mg/m^2 on day 1 plus leucovorin 400 mg/m^2, followed by an FA bolus and an FU infusion, and panitumumab 6 mg/kg on day 1, every 2 weeks). After six cycles, CT scan in September 2020 displayed disease stabilization (with no change in tumor size) and, concomitantly mild COVID-19. She recovered from COVID-19 at the end of October. Unexpected reduction of peritoneal and lung disease was reported at the CT scan following COVID-19 (Figure 1). IgG anti-SARS-CoV-2 titer by Electro-Chemi-Luminescence Immuno Assay (ECLIA) was 1216 U/ml.

Discussion

SARS-CoV-2 uses the viral spike (S) protein for host-cell attachment and entry through binding of ACE-2 (angiotensin-converting enzyme 2).[2] The host protease furin cleaves the full-length precursor S glycoprotein into two associated polypeptides: S1 and S2. Cleavage of S generates a polybasic Arg-Arg-Ala-Arg carboxyl-terminal sequence on S1 that binds to cell-surface neuropilin-1 (NRP-1) and NRP-2 receptors. Blocking this interaction by ribonucleic acid interference or selective inhibitors reduces SARS-CoV-2 entry and infectivity in cell culture.[3,4] Although wide distribution of ACE-2 across human tissues, including lung, liver, stomach, ileum, colon, and kidney was reported,[4] alveolar pneumocytes type 2, the SARS-CoV-2 main target cell, actually expressed rather low levels of ACE-2.[4–6] Hence, the SARS-CoV-2 may depend on co-receptor or other auxiliary membrane proteins to facilitate its infection. NRP-1 could represent such an ACE-2-potentiating factor by promoting the interaction of the virus with ACE-2. NRP-1 is expressed in various tissues of the body.[7–9] Thus, we can hypothesize that the SARS-CoV-2 may infect ACE-2/NRP-1-expressing colon-cancer cells, evoking a direct immune response against the infected cells. Moreover, SARS-Cov-2 binding to ACE-2/NRP-1 in CRC cells might provoke cytokine release, which could enable attracting tumor microenvironment (TME) immune cells: this could be sufficient to explain an anti-tumor effect. Furthermore, cross-reactivity with viral antigens may also be involved in T (*via* T-cell receptor interactions) or NK (natural killer) lymphocytes [*via* ADCC (antibody-dependent cellular cytotoxicity)] activation against CRC cells. However, the natural immunity lymphocytes (including NK cells), which are non-major-histocompatibility-complex restricted, quickly react toward transformed or antibody-targeted cells (killing *via* ADCC). SARS-Cov-2 resolution is likely due to the presence of antibodies, which concomitantly eliminate infected cells (*via* ADCC) and prevent virus entry into new target cells. Further study of the SARS-Cov-2-infected TME (programmed cell-death protein-1/programmed cell-death ligand-1 pathway, tumor-infiltrating lymphocytes (TILs), tumor-associated macrophages, myeloid-derived suppressor cells, intratumoral cytokines patterns, etc.) will be crucial to understand (a) the consequences of eventual viral replication into tumor cells expressing high ACE-2, and (b) the involved lymphocyte sub-populations. In this regard, as a supporting hypothesis, Patients 1, 2, and 3 convincingly expressed ACE-2 [Figure 2, panel (a)] and patients 1 and 3 isolated NKs, displaying higher

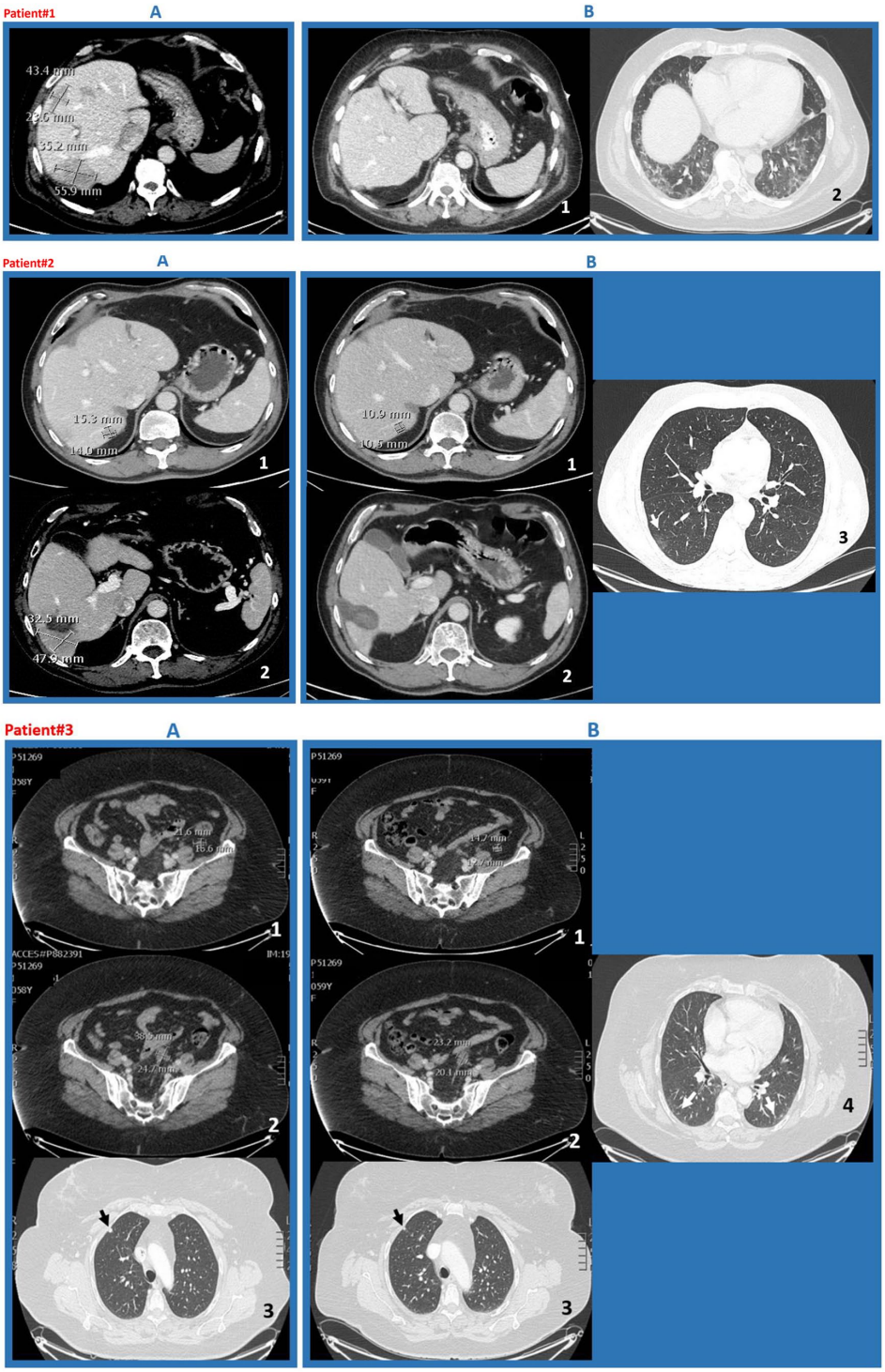

Figure 1. CT scans of patients 1–3.
Patient 1 (a) CT scan shows multiple metastatic liver lesions with relative measures (August 2020); (b) CT scan shows complete regression of metastases (November 2020); (c) persistent diffuse interstitial thickening, coarse reticular patterns, and parenchymal bands that were SARS-CoV-2 associated (November 2020). **Patient 2** (a)(i) and (ii) CT scan show metastatic liver lesions at VI and VII segments with the relative measures (August 2020); (b)(i) and (ii) CT scan shows reduction of lesion at VII segment and disappearance of that at VI segment (December 2020); (iii) some persistent interstitial thickening that was SARS-CoV-2 associated (December 2020). **Patient 3** (a)(i) and (ii) CT scan evidencing multiple measurable nodules on the peritoneum surface; and (iii) a subpleural small nodule in right upper lobe (September 2020); (b)(i) and (ii) reduction of peritoneal and (iii) lung disease; (iv) some persistent interstitial thickening that was SARS-CoV-2 associated (October 2020). CT, computed tomography; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

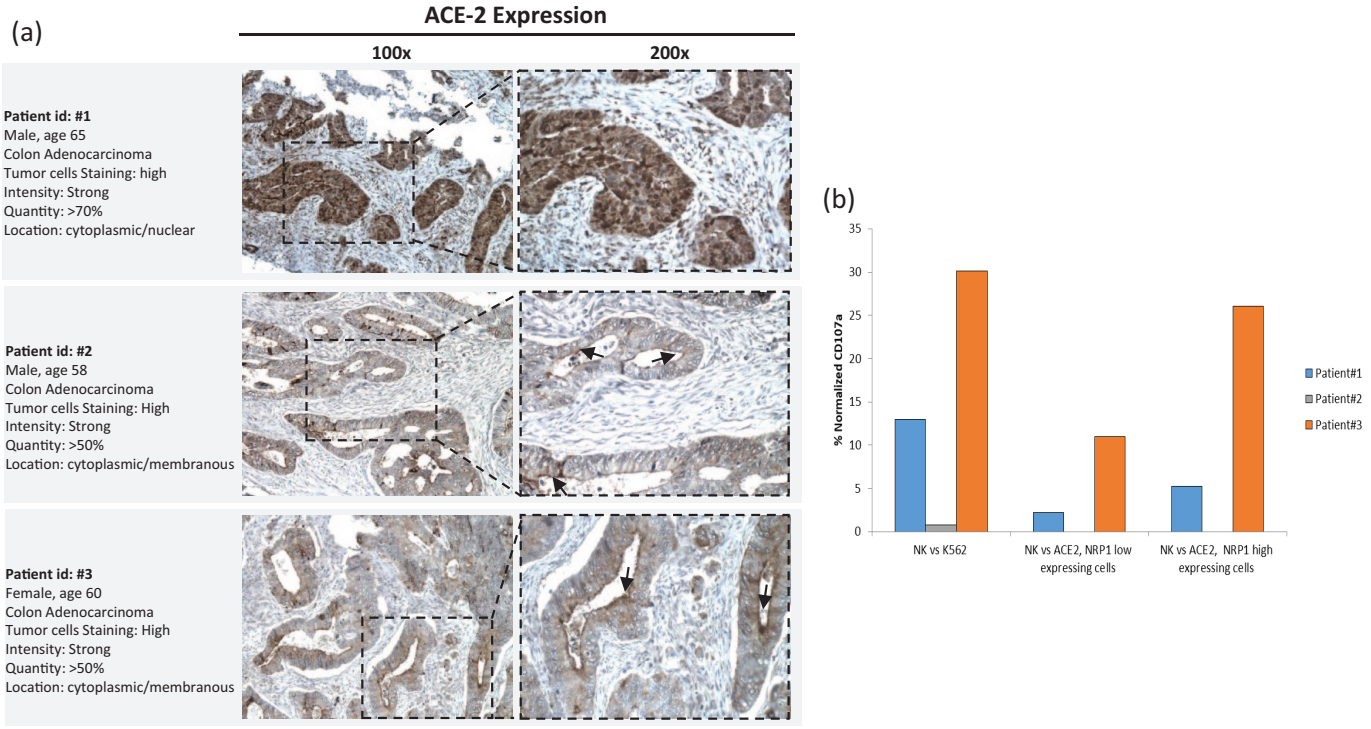

Figure 2. Representative whole section IHC staining of ACE-2, and patients' peripheral NKs display higher degranulation toward ACE-2/NRP-1-expressing cells.
(a) Representative whole section IHC (immunohistochemistry) staining of ACE-2. Staining was done with HPA000288 Sigma-Aldrich, 1:250 dilution, HIER pH9. **Patient 1** showed strong, diffuse cytoplasmic/nuclear staining for ACE-2 in colonic cancer cells. **Patient 2** showed strong, diffuse cytoplasmic/membranous ACE-2 expression, with apical linear staining pattern, to seal the apical intercellular spaces of glandular epithelia and interfacing to the paracellular macromolecules (arrow). **Patient 3** showed strong and diffuse cytoplasmic/membranous ACE-2 expression with apical linear staining pattern (arrow). (b) Patients peripheral NKs display higher degranulation toward ACE-2-/NRP-1-expressing cells. HCT 116, human colon cancer cells, were low ACE-2 and NRP-1 expressing; IGROV-1, human ovarian cancer cells, were high ACE-2 and NRP-1 expressing (Tumor cell lines were puchased from American Tissue Cultutre Collection, www.lgcstandards-atcc.org). Purified NK cells (0.1×10^6) from patients' peripheral blood were incubated with K562 cells (E:T ratio 10:1) and degranulation was evaluated through the lysosomal protein LAMP-1 (CD107a-PE, Clone H4A3, BD Pharmingen™). Patient 1 and patient 3 NKs displayed higher activity toward ACE-2-positive/NRP-1-positive-cells.
ACE-2, angiotensin-converting enzyme 2; E, effector; NK, natural killer cells; NRP, neuropilin; T, target.

degranulation [Figure 2, panel (b)] toward ACE-2-/NRP-1-positive cells.

In the period September–October 2020, 60 mCRC patients were observed. Interestingly, seven patients, not included in this report, developed COVID-19, and recovered from it in a median time of 34 days. In February 2021, four patients displayed undetectable humoral response (IgG <0.04 U/ml) and three showed IgG 0.8, 1.1, 3.6 U/ml. Although a detailed clinical–pathological description of this series is beyond the scope of this report, these patients did not show tumor burden reduction.

A characterization of TILs in metastatic tissue is lacking in our report. However, in CRC, there is a strong and repeatable association between microsatellite instability high (MSI-high) and abundant infiltration by CD3+ T cells. Nevertheless, the described patients were MSS. This may suggest that the observed phenomenon is more likely linked to effector cells from innate immunity.

Interestingly, a previous report linked SARS-CoV-2 infection with neoplastic course in Hodgkin lymphoma.[10] Here, we described, for the first time, improvement in mCRC disease in three patients undergoing COVID-19. Our observation may contribute to generate hypotheses on the infection mechanism of the SARS-CoV-2 virus and the multiple aspects of a composite antiviral immune response in cancer patients.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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