Abstract. The present case report describes a case of left renal clear cell carcinoma with brain, lung, para-aortic, and lymph node metastases (cT1bN1M1) in a 52-year-old Japanese male. The patient received sequential anticancer treatments with pazopanib, everolimus, and axitinib, but exhibited treatment-resistant tumor growth. Treatment with nivolumab resulted in a complete response in metastatic sites. However, the residual renal tumor, which was enhanced by contrast medium, required radical nephrectomy. Pathological analyses of the renal tumor revealed that it consisted of fibrotic and lymphocyte-infiltrated tissues in which morphological cancer cells were not detected. The majority of lymphocytes were cluster of differentiation (CD)8-positive, suggesting that cancer cells were attacked by these lymphocytes. Retrospective analyses of renal cell carcinoma tissues, which were biopsied before the anticancer treatment, revealed their infiltration by CD8-positive T cells. To the best of our knowledge, this is the first case report to examine renal tissue prior to and following treatment with nivolumab using immunohistochemical analysis.

Introduction

Treatments using nivolumab, a humanized antibody targeting programmed cell death protein 1 (PD1), achieve durable effects in approximately 15% of patients with metastatic renal cell carcinoma (mRCC) (1-4). Choueiri et al (5) reported immunomodulatory activity of nivolumab in biopsied mRCC tissues, which were biopsied before the anticancer treatment, revealed their infiltration by CD8-positive T cells. To the best of our knowledge, this is the first case report to examine renal tissue prior to and following treatment with nivolumab using immunohistochemical analysis.
treat brain metastasis, the administration of pazopanib was temporarily stopped 9 days after its initiation (on Day #9), and stereotactic radiotherapy was performed using the Cyber Knife G3 machine (Accuray Inc., Sunnyvale, CA, USA) on Days #10, 11 and 13 (total 35 Gy, ‘ck’ in Fig. 1A). The administration of pazopanib was resumed on Day #18. The brain metastasis gradually shrank to 8.6±0.2 mm on Day #58 (MRI #2; Fig. S2B), to 1.6±1.6 mm on Day #154 (MRI #3; Fig. S2C), and disappeared after Day #422 (MRI #4-5; Fig. S2D and E). The complete response to treatment observed for brain metastasis can presumably be attributed to radiotherapy and/or pazopanib (‘brain’ in Fig. 1A). Similarly, the two lung lesions disappeared under pazopanib treatment (‘lt. lung 1’ and ‘lt. lung 2’ in Fig. 1A, respectively; 3rd and 5th columns of Fig. S1, respectively). The renal tumor and para-aortic lymph node continued to shrink during the course of the treatment with pazopanib (‘renal tumor’ and ‘para Ao’ in Fig. 1A, respectively). De novo lung metastasis was detected in the lower lobe of the right lung (‘rt. lung’ in Fig. 1A; 4th column of Fig. S1). Therefore, the molecular targeted agent was altered from pazopanib to everolimus on Day #335. The sizes of the renal tumor, para-aortic lymph node, and right lung metastasis did not significantly decrease between CT #5 (Day #307) and CT #7 (Day #462) (P>0.05, unpaired Student's t-test); therefore, everolimus was switched to axitinib. The treatment with axitinib was not effective because the sizes of the renal tumor and right lung metastasis increased from CT #7 (Day #462) to CT #8 (Day #539) (P=0.0062 and 0.0051, respectively) (Fig. 1A and S1). Four metastatic lesions (the brain, lt. lung #1, lt. lung #2, and para-aortic lymph node in Fig. 1A and S1) were controlled by the treatments described above, while de novo metastasis in the right lung (Fig. 1B) and the renal tumor with apparent contrast enhancement (Fig. 1C) required further anticancer treatment.

**Immunotherapy using nivolumab (4th).** A bi-weekly treatment with 207 mg (3 mg/kg) of nivolumab, which was approved for the treatment of mRCC in Japan at around that time, was initiated on Day #563. After 5 courses of the treatment with nivolumab, de novo lung metastasis markedly decreased in size [from 20.4±1.1 mm (CT #8; Fig. 1C and S1) to 6.2±0.6 mm (CT #9; Fig. 1E and S1), P=0.0003]. The size of the unenhanced portion of the renal tumor remained unchanged (‘ in Fig. 1C and E), suggesting that it was necrotic tissue before treatment with nivolumab. The portion enhanced by contrast medium significantly contracted to a small mass with weak enhancement (pink arrowheads in Fig. 1C and E), suggesting that the primary lesion contained a small number of viable cancer cells.

**Radical nephrectomy.** The patient underwent left laparoscopic nephrectomy on Day #728 without perioperative complications; however, it was not possible to remove the para-aortic lymph node. The pathological findings described below revealed no viable tumor cells, leading to the diagnosis of a pathological complete response. The patient resumed treatment with nivolumab because we are not able to clearly estimate the timing for discontinuation, and neither recurrence nor de novo metastasis including para-aortic lymph node was detected on Day #836 (Fig. 1A).

**Pathological findings.** The tumor was whitish with brown central necrosis in a coronal section of the extracted kidney (green arrowheads in Fig. 2A and B). A normal portion in block #4 (one of the large number of tissue blocks prepared for a pathological diagnosis, ‘ in the left side of Fig. 2B) was histologically normal (‘ in Fig. 2C). Cancer cells were not detected in the tumor, into which a large number of CD8-positive T cells had infiltrated (“ in Fig. 2C and D).
The immunohistochemical staining shown in Fig. 2D was performed using a mouse monoclonal anti human CD8 antibody (clone C8/144B; Dako, Santa Clara, CA, USA). Another tissue block (block #5 in the right side of Fig. 2B) showed similar findings in normal tissue [* in Fig. 2E and F] and tumor tissue [# in Fig. 2E and F] in block #5, respectively. Scale bars in panels C-F indicate 5 mm. [* in panels C and D] correspond to those in the left-hand tissue of panel (B). [# and # in panels (E and F)] correspond to those in the left-hand tissue of panel (B). (G and H) High magnification images of portions indicated by yellow arrowheads in panels (E and F), respectively (renal tumor portion in which a large number of CD8-positive T cells had infiltrated). (I and J) High magnification images of portions indicated by orange arrowheads in panels (E and F), respectively (fibrotic tissue in which a few T cells had infiltrated). Scale bars in panels (G-J) indicate 100 µm. (K) Low magnification images of H&E staining of biopsied specimens. (L-N) Immunohistochemical staining images for CD8, PD1 and CD4 using serial sections of the coronal section in panel (K). Scale bars in panels (K-N) indicate 1 mm. [* and arrowheads (yellow and green) indicate degenerated renal tissue and renal carcinoma tissue, respectively. (O-S) High magnification images of the portion indicated by the yellow and green arrowheads in panels (K-N), respectively. Scale bars in panels (O-S) indicate 100 µm. H&E, hematoxylin and eosin; CD, cluster of differentiation; lt, left; rt, right; PD1, programmed cell death protein 1. In summary, the pathological findings of the yellow arrowheads in Fig. 2E and F) as previously reported (7). The inner tissue of the tumor was completely occupied by fibrotic tissue, into which only a few T cells had infiltrated [# the right side of Fig. 2B, E and F, as well as Fig. 2I and J (enlarged image of the orange arrowheads in Fig. 2E and F)].
in this case exhibited the effects of nivolumab on RCC as follows: i) No impact on normal renal tissue (no T cell infiltration); ii) significant T cell infiltration into presumably viable tumor tissue (’ in Fig. 2B-F, 2G and H); and iii) fibrosis after presumable necrosis and/or phagocytosis after T cell infiltration induced by nivolumab (# in Fig. 2B, E and F as well as Fig. 2I and J).

Discussion

In 2014, Tumeh et al (8) reported that melanoma tissues infiltrated with many CD8-positive T cells are associated with a good response to therapy using pembrolizumab, an immune checkpoint inhibitor targeting the PD-1, PD-L1 axis (termed adaptive immune resistance (4,9). In these tissues, the number of CD8-positive T cells is significantly increased by pembrolizumab, leading to melanoma cell injury. Similar histological findings may be observed in RCC tissues before and after treatments using nivolumab; however, to our knowledge, a comparison of RCC tissues before and after treatment with nivolumab was not yet conducted.

We retrospectively analyzed biopsy specimens (on Day #–4) obtained from the patient using the same anti-CD8 antibody, mouse monoclonal anti-human PD1 antibody (NAT105; Abcam, Tokyo, Japan), and mouse monoclonal anti-human CD4 antibody (clone 1F6; Novocastra, Tokyo, Japan) (Fig. 2K-N). The specimen consisted of degenerated non-cancer renal tissues (’ in Fig. 2K) and clear cell carcinoma tissues (yellow and green arrowheads). Carcinoma tissues were infiltrated by a large number of T cells (Fig. 2P), which expressed CD8 (Fig. 2L), PD1 (Fig. 2M), and CD4 (Fig. 2N) at different levels. Fig. 2P-S show the carcinoma tissue portion that expressed CD8 at the highest level within the biopsy specimen. Even in that portion (Fig. 2Q), the number of CD8-positive T cells was markedly lower than in the tumor portion of nephrectomized tissue (Fig. 2H). T cells in the tumor portion more weakly expressed PD1, suggesting that most PD1-positive carcinoma cells were attacked by a higher number of CD8-expressing T cells. The clinical course and pathological findings described above imply that CD8 immunohistochemistry for biopsied specimens of RCC tissue may potentially predict the effectiveness of nivolumab, as reported previously by Tumeh et al (8) in melanoma tissues.

We herein report a case of mRCC that achieved a complete response with nivolumab using detailed histochemical (H&E) and immunohistochemical analyses. The number of CD8-expressing T cells in biopsied specimens may play a role in predicting the treatment effects of nivolumab, as previously reported for pembrolizumab in melanoma tissue. Further investigations are needed in order to confirm this hypothesis from a single case experiment.

This is the first case report to show that nivolumab contributed to achieving a histological as well as radiological complete response in a patient with multiple metastatic RCC who had not responded to other molecular targeted therapies.

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Availability of data and materials

Figures S1 and S2 are available in the Figshare repository. Figure S1, CT imaging during clinical course: doi.org/10.6084/m9.figshare.7063880.v1; Figure S2, MRI showing brain metastasis during clinical course: doi.org/10.6084/m9.figshare.7063922.v1.

Authors’ contributions

SS and GK planned the case study, drafted the manuscript, and analyzed the patient data. KojN performed immunohistochemical examination and interpreted the pathological findings. MO and KosN accurately interpreted the case study and critically revised this manuscript for important intellectual content. All authors read and approved the version to be published.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

The patient provided written informed consent for the publication of this case report.

Competing interests

The authors declare that they have no competing interests.

References


