Case Report

Bladder urothelial carcinoma producing insulin-like growth factor II: A case report

Satoshi Funada,[1,†] Yuki Kita,[1,†] Yoshiyuki Okada,[1] Takashi Kobayashi,[1] Yuki Teramoto,[2] Shinsuke Shibuya,[2] Ryoichi Saito,[1] Kaoru Murakami,[1] Keiyu Matsumoto,[1] Feng Yang,[3] Dimiter S Dimitrov,[3] Takahiro Inoue[1] and Osamu Ogawa[1]

Departments of [1]Urology, and [2]Diagnostic Pathology, Kyoto University Graduate School of Medicine, Kyoto, Japan, and [3]Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute, Frederick, Maryland, USA

Abbreviations & Acronyms
AKT = serine/threonine protein kinase
CT = computed tomography
GH = growth hormone
H&E = hematoxylin and eosin
IGF = insulin-like growth factor
NICTH = non-islet cell tumor hypoglycemia
PDX = patient-derived xenograft
RECIST = Response Evaluation Criteria in Solid Tumors
TUR = transurethral resection

Correspondence: Osamu Ogawa M.D., Ph.D., Department of Urology, Kyoto University Graduate School of Medicine, 54 Shogoinkawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan. Email: ogawao@kuhp.kuoyo-u.ac.jp

Received 25 June 2018; accepted 25 July 2018. Online publication 20 September 2018

†These authors contributed equally to this work.

Introduction: Non-islet cell tumor hypoglycemia is a rare paraneoplastic syndrome associated with tumors. Although it mainly occurs in solid tumors of mesenchymal and epithelial origin, but rarely also in hematopoietic and neuroendocrine origin.

Case presentation: We describe a 65-year-old man with a muscle-invasive bladder urothelial carcinoma, which rapidly progressed against systemic chemotherapy consisting of gemcitabine and cisplatin. Notably, the patient developed hypoglycemia at the terminal stage of the disease. Pathological diagnosis was giant cell urothelial carcinoma, which was strongly positive for insulin-like growth factor-II in immunohistochemistry. We established patient-derived xenograft from insulin-like growth factor-II producing bladder urothelial carcinoma that caused non-islet cell tumor hypoglycemia. Although we evaluated the efficacy of the neutralizing antibody, there was no statistically significant inhibitory effect on tumor growth.

Conclusion: To the best of our knowledge, this is the first report of insulin-like growth factor-II-producing urothelial carcinoma that have been recapitulated in a patient-derived xenograft model.

Key words: animal model, bladder cancer, hypoglycemia, IGF-II, patient-derived xenograft.

Keynote message

This is the first report described bladder urothelial carcinoma producing IGF-II and inducing NICTH. We experienced a 65-year-old man with a muscle-invasive bladder urothelial carcinoma developed hypoglycemia, diagnosed as NICTH. We have successfully established PDX from IGF-II producing bladder urothelial carcinoma, which caused NICTH. Although we evaluated the efficacy of the neutralizing antibody, there was no statistically significant inhibitory effect on tumor growth.

Introduction

NICTH is a rare paraneoplastic syndrome associated with a variety of benign and malignant tumors. Although it mainly occurs in solid tumors of mesenchymal and epithelial origin, but rarely also in hematopoietic and neuroendocrine origin.[1] Clinically, NICTH is characterized by hypoglycemia, low insulin and c-peptide, low growth hormone, IGF-I and IGFBP3, and high molecular weight ("big") IGF-II in patient sera.[2] The ideal management of NICTH is total resection of the IGF-II-producing tumor, which is not always feasible.[2]

In the present case, we established PDX from IGF-II producing bladder urothelial carcinoma that caused NICTH and evaluated the efficacy of the neutralizing antibody.

Clinical summary

A 65-year-old man presented with macroscopic hematuria. Cystoscopy and contrast-enhanced CT revealed a solid and sessile bladder tumor without regional lymph node

involvement or distant metastasis (Fig. 1a). Pathological diagnosis on TUR was muscle-invasive high-grade urothelial carcinoma. When the patient was referred to our hospital, the tumor had rapidly progressed and extravesical extension was revealed on CT (cT3bN0M0, Fig. 1b) and the patient received five courses of systemic administration of gemcitabine (1000 mg/m^2 on days 1 and 8) plus cisplatin (60 mg/m^2 on day 2). Although he showed maximum objective response of 46% (Fig. 1c) according to RECIST (version 1.1), the tumor eventually progressed with peritoneal dissemination (Fig. 1d). At the same time, the patient developed hypoglycemia, which was persistent after discontinuation of the drugs. Endocrinological evaluation revealed normal adrenal function, suppressed levels of insulin (<0.5 μU/mL, reference range: 3–15), c-peptide (0.07 ng/mL, reference range: 0.69–2.45), GH (<0.07 ng/mL, reference range: <2.1), and IGF-I (73 ng/mL, reference range: 72–221). Along with bladder cancer progression, the hypoglycemia was exacerbated and the plasma glucose level was hardly maintained despite treatment with corticosteroid and continuous glucose infusion (Fig. 1e). The patient died 8 months after the initial presentation.

Pathologic findings

On postmortem examination, the pelvic cavity was occupied by large mass (11.8 cm in diameter) and the peritoneal cavity was filled with bloody ascites. Numerous tumor dissemination was observed on peritoneum, mesentery and omentum.

Microscopic examination showed highly bizarre pleomorphic tumor cells admixing with numerous giant cells arranged in single-file pattern or diffuse discohesive pattern-less architecture in a loose myxoid stroma (Fig. 2). Immunohistochemistry revealed that tumor cells including multinucleated giant cells were strongly positive for IGF-II (antibody: ab9574; Abcam, Cambridge, UK) (Fig. 2), whereas giant cells were negative for CD68 (M0876; DAKO, Glostrup, Denmark) and beta–human chorionic gonadotropin (A0231; DAKO). Final pathological diagnosis was giant cell urothelial carcinoma. Intriguingly, the pretreatment primary tumor was also positive for IGF-II. Based on these findings, we concluded that the patient developed NICTH caused by bladder urothelial carcinoma producing IGF-II.

After written informed consent had been obtained from patient family, a 10-mm fragments of primary bladder tumor was implanted to two severe combined immunodeficient mice (CB-17/Icr-*Prkdc*^{scid}/CrlCrlj) 6 h after death as described elsewhere.[3] The mice were housed and cared for in compliance with a protocol approved by Institutional Animal Care and Use Committee at Kyoto University Graduate School of Medicine. The implanted tumor was successfully engrafted in the two mice (take rate: 100%) and started growing in 3 weeks. The established PDX showed similar histological characteristics to the pretreatment and post-treatment original tumors, and

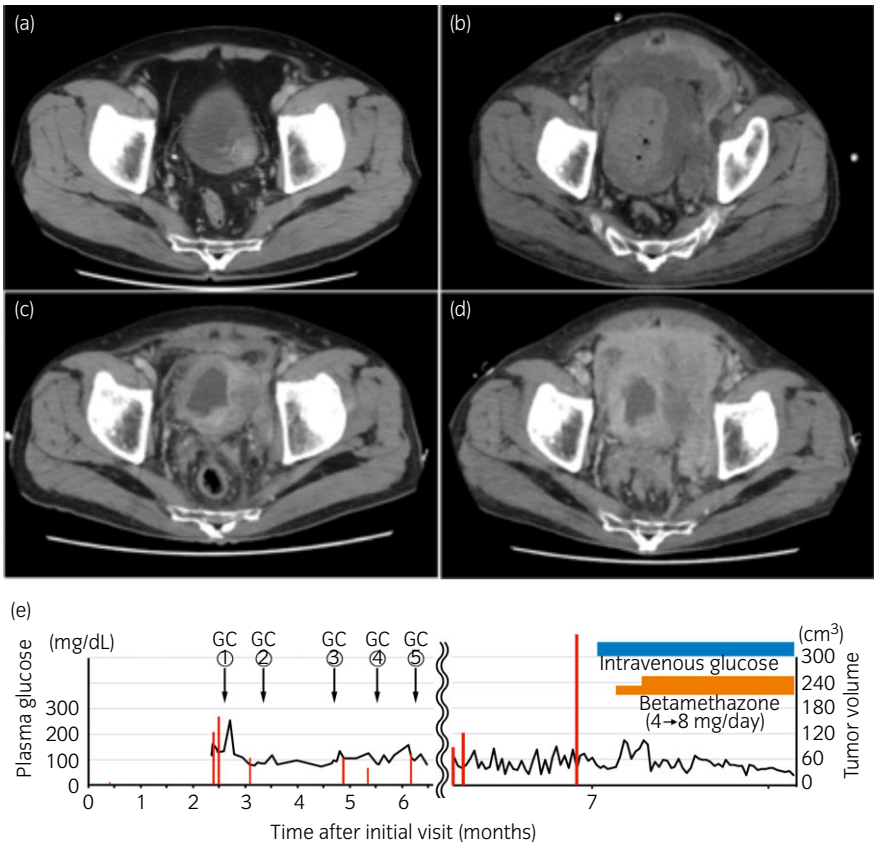

Fig. 1 (a–d) Contrast-enhanced CT scans at initial visit (a), pre- (b) and post- (c) chemotherapy settings, and progression (d). (e) Clinical course with regard to plasma glucose (black line) and tumor volume (red columns) from the initial visit to decease. Note the rapid tumor progression and severe hypoglycemia resistant to intravenous glucose injection and betamethasone administration. Tumor volume was estimated using the following formula; A2 × B × 0.53, where A and B indicate the largest and orthogonal diameter, respectively, in axial image of CT scan.

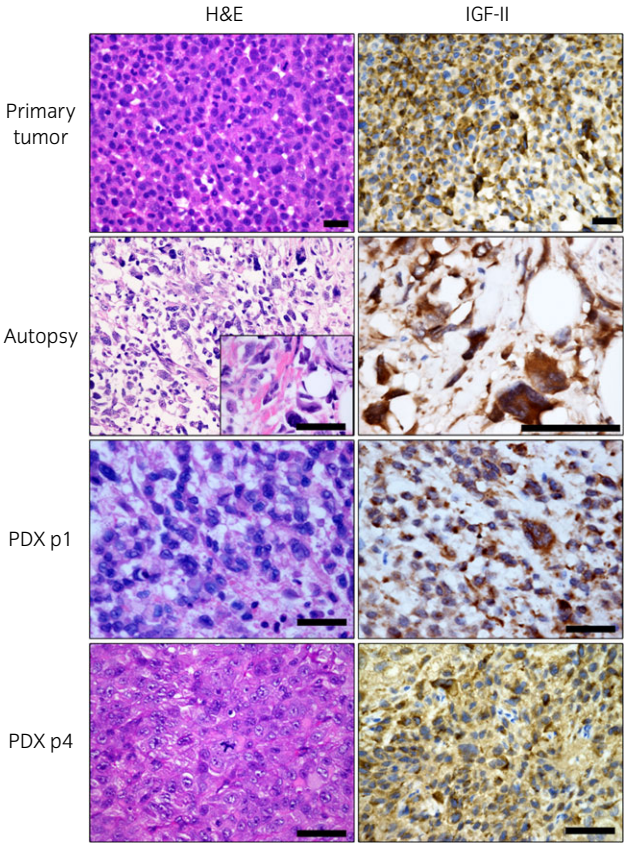

Fig. 2 Microphotographs of H&E stain (top) and immunohistochemical stain for anti-IGF-II antibody on pretreatment, autopsied, 1st- (p1) and 4th- (p4) passage PDX tumors. Scale bars indicate 50 μm.

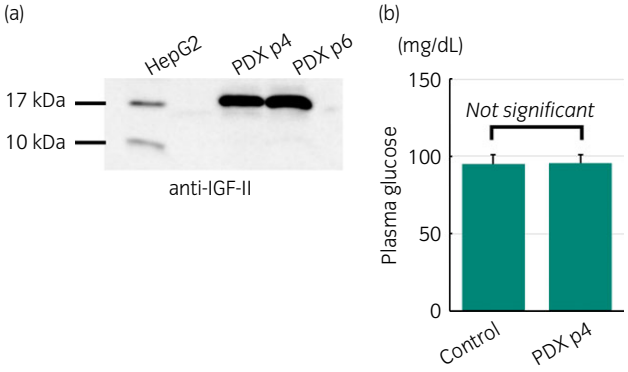

Fig. 3 (a) Western blotting showing PDX tumors express high molecular weight ("big") IGF-II. HepG2 cell acts as positive control that expresses normal and "big" IGF-II. (b) Plasma glucose levels of intact (control) and tumor-bearing (PDX p4) mice (n = 3 each). There was no significant statistical difference between the two groups (Student t-test).

importantly, preserved IGF-II expression (Fig. 2). Western blotting revealed that IGF-II expressed by PDX tumors was mostly high molecular weight ("big") variant, as evident by comparison with HepG2 cells that were reported to express both normal and "big" IGF-II (Fig. 3a).[4] Nonetheless, tumor-bearing mice did not show any sign suggesting hypoglycemia or abnormal plasma glucose levels (Fig. 3b).

When PDX tumors reached 10 mm in diameter, the tumor-bearing mice were randomly assigned and treated with weekly intraperitoneal administration of a neutralizing anti-IGF-II antibody[5] or control IgG (10 mg/kg) for 4 weeks. Tumors on neutralizing antibody-treated mice did not show significant growth retardation compared with those on control IgG-treated mice (Fig. 4a). Phosphorylation levels of AKT, which is known as downstream target of activated IGF receptor,[6] was suppressed in those treated with anti-IGF-II antibody compared with those treated with control IgG (Fig. 4b). The PDX line was successfully maintained until the tumor growth rate gradually became higher and all tumor-bearing mice died soon after the ninth passages.

Discussion

The present case harbored most of the above-mentioned characteristics of NICTH including strong immunohistochemical staining of IGF-II in the patient tumor tissues. However, we could not confirm expression of high molecular weight IGF-II in tumor tissue or serum of the patient. Instead, we detected it in PDX tumor and thereby confirmed the diagnosis of NICTH in the present case. To the best of our knowledge, this is the first description of a case of IGF-II-producing bladder urothelial carcinoma associated with NICTH, although a few cases of NICTH caused by solitary fibrous tumor of the bladder have been reported.[7]

The PDX model established in the present study preserved histological characteristics of original tumor as well as strong IGF-II expression, which helped better understanding of this rare pathogenic entity. PDX models recently attract considerable attention[3] particularly for the researches on certain cancers that have been hampered by the shortage of available animal models.[8]

We have successfully demonstrated the feasibility and usefulness of the IGF-II-producing PDX as a preclinical model. Although treatment with an anti-IGF-II neutralizing antibody failed to show statistically significant inhibitory effect on tumor growth, we could additionally show marked suppression of IGF-II and phosphorylated AKT expression. This indicated that anti-IGF-II neutralizing antibody successful blocked IGF-II and a downstream signaling pathway but the tumor cells were not completely dependent on them, although we did not test whether the neutralizing antibody bound to tumor-derived IGF-II. As for IGF-II-induced hypoglycemia, PDX-bearing mice did not reproduce clinical characteristics of NICTH despite strong IGF-II expression by PDX tumor. This might be caused by little affinity of human IGF-II to mouse insulin receptor.

Giant cell urothelial carcinoma is a rare variant of infiltrating urothelial carcinoma.[9] It is characterized by the presence of large bizarre anaplastic mononuclear cells and multinucleated giant cells[10] and with a higher probability of lymph node involvement and distant metastasis.[11] Although extrapulmonary primary carcinomas with giant cell morphology have been described in the other organs,[12] there has been no report associated with NICTH.

In summary, we have reported the first described case of bladder urothelial carcinoma producing IGF-II and inducing

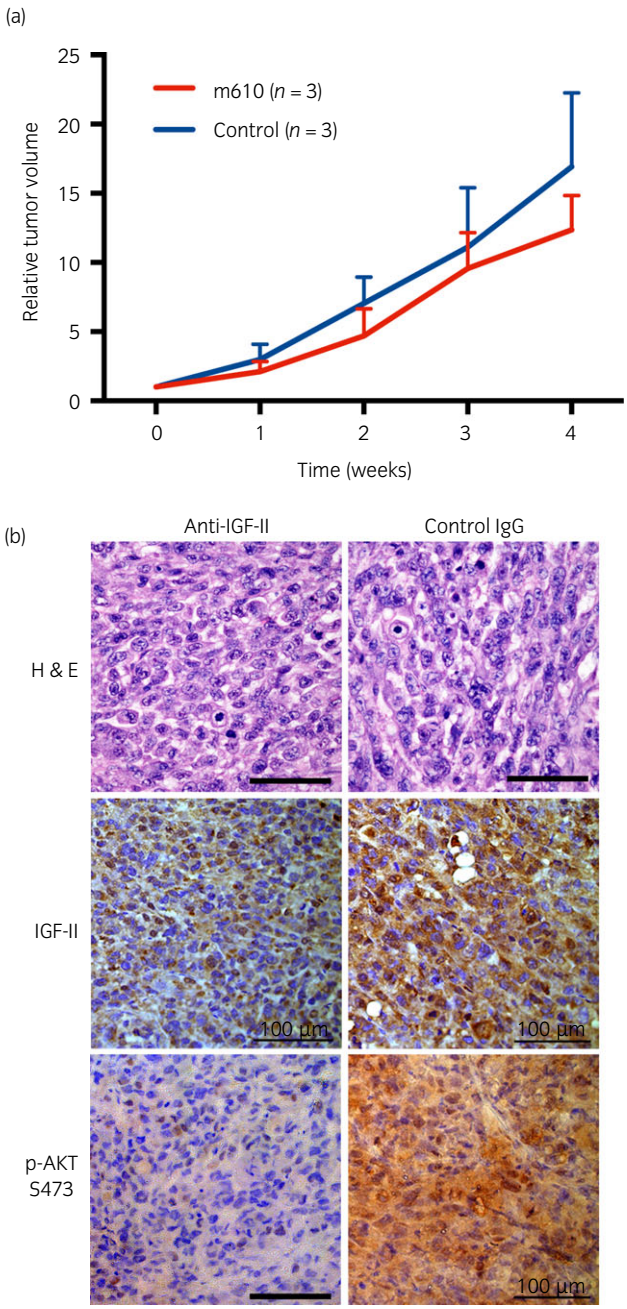

Fig. 4 (a) Relative growth curves of PDX tumors treated with anti-IGF-II neutralizing antibody (m610) or control IgG (Control) (n = 3 each). (b) Microphotographs of H&E stain, and immunohistochemical stains for anti-IGF-II and phosphorylated AKT (S473; Cell Signaling Technology, Danvers, MA, USA #9271) on PDX tumors treated with anti-IGF-II neutralizing antibody and control IgG. Scale bars indicate 100 µm.

NICTH. We have successfully established PDX, which showed potential usefulness for precise diagnosis, preclinical testing of promising drugs, and investigation of molecular pathogenesis and drug resistance of this rare disease entity.

Acknowledgments

The authors thank the patient family for providing written informed consent to the publication of the clinicopathological details. The authors are also grateful to Dr Hiroshi Suzuki at the Department of Urology, Sugita Genpaku Memorial Obama Municipal Hospital, Obama, Fukui, Japan for providing detailed clinicopathological information. This work was partly supported by Grant-in-Aid for Young Scientist (A) from the Japanese Society for the Promotion of Science (#25713055, to T.K.) and the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research (to D.S.D.).

Conflict of interest

The authors declare no conflict of interest.

References

1 de Groot JW, Rikhof B, van Doorn J *et al.* Non-islet cell tumour-induced hypoglycaemia: a review of the literature including two new cases. *Endocr. Relat. Cancer* 2007; **14**: 979–93.
2 Bodnar TW, Acevedo MJ, Pietropaolo M. Management of non-islet-cell tumor hypoglycemia: a clinical review. *J. Clin. Endocrinol. Metab.* 2014; **99**: 713–22.
3 Inoue T, Terada N, Kobayashi T, Ogawa O. Patient-derived xenografts as in vivo models for research in urological malignancies. *Nat. Rev. Urol.* 2017; **14**: 267–83.
4 Bae MH, Lee MJ, Bae SK *et al.* Insulin-like growth factor II (IGF-II) secreted from HepG2 human hepatocellular carcinoma cells shows angiogenic activity. *Cancer Lett.* 1998; **128**: 41–6.
5 Feng Y, Zhu Z, Xiao X, Choudhry V, Barrett JC, Dimitrov DS. Novel human monoclonal antibodies to insulin-like growth factor (IGF)-II that potently inhibit the IGF receptor type I signal transduction function. *Mol. Cancer Ther.* 2006; **5**: 114–20.
6 Nunes M, Vrignaud P, Vacher S *et al.* Evaluating patient-derived colorectal cancer xenografts as preclinical models by comparison with patient clinical data. *Cancer Res.* 2015; **75**: 1560–6.
7 Schutt RC, Gordon TA, Bhabhra R *et al.* Doege-Potter syndrome presenting with hypoinsulinemic hypoglycemia in a patient with a malignant extrapleural solitary fibrous tumor: a case report. *J. Med. Case Rep.* 2013; **7**: 11.
8 Kobayashi T, Owczarek TB, McKiernan JM, Abate-Shen C. Modelling bladder cancer in mice: opportunities and challenges. *Nat. Rev. Cancer* 2015; **15**: 42–54.
9 Humphrey PA, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO classification of tumours of the urinary system and male genital organs-part B: prostate and bladder tumours. *Eur. Urol.* 2016; **70**: 106–19.
10 Samaratunga H, Delahunt B, Egevad L *et al.* Pleomorphic giant cell carcinoma of the urinary bladder: an extreme form of tumour de-differentiation. *Histopathology* 2016; **68**: 533–40.
11 Domanowska E, Jozwicki W, Domaniewski J *et al.* Muscle-invasive urothelial cell carcinoma of the human bladder: multidirectional differentiation and ability to metastasize. *Hum. Pathol.* 2007; **38**: 741–6.
12 Al-Nafussi AI. Common histological pattern and cell types of tumors: distinctive cell type. In: Al-Nafussi AI (ed). *Practical Approach and Pattern Analysis*. Hodder Arnold, London, 2005; 52.