cDNA microarray analysis assists in diagnosis of malignant intrarenal pheochromocytoma originally masquerading as a renal cell carcinoma

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Intrarenal pheochromocytoma (paraganglioma) is a very rare tumour. Its diagnosis is often difficult to establish because of its rarity and its histological similarity to renal cell carcinoma (RCC). Recently, we examined the molecular signatures of different subtypes of kidney tumours by using cDNA microarray. The signature pattern for one tumour, which was originally diagnosed as granular cell RCC, was clearly distinct from that of any other subtype of kidney tumour, and led us to re-evaluate the case. Haematoxylin and eosin staining revealed histological features suggestive of pheochromocytoma, and immunohistochemical studies showed positive staining for neuroendocrine markers but not for keratin. A germline missense mutation, D119E, in the familial paraganglioma related gene succinate dehydrogenase subunit D (SDHD), was subsequently identified. The treatment modality was revised and radiotherapy was given, to which the patient responded, leading to a reduction in tumour size of 25% within the first month. To our knowledge, this is the first report of an intrarenal pheochromocytoma that was diagnosed with the assistance of cDNA microarray analysis.

MATERIALS AND METHODS
The study was approved by the ethics committee of the University of Tokushima, Japan, and informed written consent was obtained from the patient.

Patient
A 26 year old man presented with hypertension (180/100 mmHg) in 1997. Pheochromocytoma was investigated, but no conclusive evidence was found. Plasma adrenaline and noradrenaline, cortisol, renin, aldosterone, urinary vanillylmandelic acid and homovanillic acid were within normal limits. Ultrasonography demonstrated a right renal mass. Abdominal computerised tomography revealed a solid mass in the right renal area, measuring approximately 9×5 cm (fig 1A) that contained a low density area indicating necrosis. Marked lymphadenopathy was found from the level of the coeliac arterial trunk to the level of bifurcation of the common iliac artery, with the largest lymph node exceeding 5 cm in diameter. MRI showed the same intensity as renal cortex in a T1 weighted image and showed high intensity in a T2 weighted image, suggesting necrosis in the tumour. No other lesions were found in the chest, mediastinum, or pelvis. Selective renal arteriography revealed a hypervascular tumour in the middle to lower pole of the right kidney with tumour blush in venous phase (fig 1B).

Pathological examination of a percutaneous biopsy could not confirm the diagnosis. Following embolisation of the right renal artery, a right nephrectomy and lymphadenectomy were performed via a transabdominal approach. The right adrenal gland was not involved. The patient’s blood pressure was well controlled during the surgery. Histopathologically, the tumour was diagnosed by haematoxylin and eosin staining to be a renal cell carcinoma (RCC) of the granular cells, with renal capsular penetration and lymph node metastases (fig 2A). Postoperatively, the patient was normotensive and his "RCC" was treated with 5×10⁶ units of interferon-α, cimetidine, and tegafu uracil. Ten months after the operation, enlarged para-aortic lymph nodes were detected and then resected. The resected lymph node was also histopathologically diagnosed as metastases of granular cell RCC.

cDNA microarray findings
In 2001, we were analysing the gene expression profiles of seven different histological subtypes of kidney tumours to determine if these subgroups can be distinguished by their gene expression profiles using microarrays containing 19 968 cDNAs. In total, 70 kidney tumours (including 39 clear cell RCCs, one metastatic and seven primary papillary RCCs, six...

Abbreviations: AML, acute myeloid leukaemia; MIBG, metaiodobenzylguanidine; RCC, renal cell carcinoma; SDH, succinate dehydrogenase
granular cell RCCs (including the present case), five chromophobe RCCs, two sarcomatoid RCCs, two oncocytomas, three transitional cell carcinomas (TCCs) of the renal pelvis, and five Wilms tumours) were compared with noncancerous kidney tissues. Based on the expression patterns of 3560 selected cDNAs, we found distinct molecular signatures in the clear cell, papillary, chromophobe RCC/oncocytoma, TCC, and Wilms subtypes, whereas the six cases with granular cell RCC did not show a common signature (fig 3).

Pathological and immunohistochemical evaluation of “granular cell RCC”
These “granular RCC” cases were further reviewed histologically by a urological pathologist (XJY), blinded to the study. All granular cell RCC cases except the present case were reclassified as clear cell RCC (n = 4) or oncocytoma (n = 1), which were compatible with gene expression classification. The present case, which was not clustered with any subtype of RCC, was also reevaluated using NSE, HMB45, synaptophysin, S100, and keratin immunohistochemistry (performed as previously described).

Mutation analysis of succinate dehydrogenase genes
The genes encoding the mitochondrial complex II subunits SDHA, SDHB, SDHC, and SDHD, were screened for mutations as previously reported. PCR amplification was carried out in 1× PCR buffer (Qiagen) containing 200 μmol/l deoxynucleotide triphosphate, 0.6 μmol/l of each primer, 2.5 U Taq polymerase (Qiagen), and 100–200 ng of tumour DNA template in a 50 μl volume. PCR conditions were one cycle of 15 minutes at 95°C then 35 cycles of 1 minute at 95°C, 1 minute at 58°C, and 1 minute at 72°C, which was followed by one cycle of 10 minutes at 72°C.

PCR amplicons were purified by gel electrophoresis (Bio-Rad Laboratories, Hercules, CA) and column purification (Wizard PCR Prep; Promega, Madison, WI), and subjected to semiautomated sequencing using the aforementioned primers with a dye terminator method, and sequenced on an ABI377xl or PE3700 sequencer. The sequencing was repeated on DNA of the matched normal kidney.

RESULTS
Histology showed clusters of tumour cells with granular cytoplasm and prominent vascularity, suggestive of pheochromocytoma, extensively infiltrating the kidney parenchyma (fig 2A, B). Further immunohistochemical analysis showed positive staining for NSE, HMB45, and synaptophysin (focally positive) and negative staining for keratin in cancer cells, and positive staining for S100 in sustentacular cells (fig 2–E). These findings further support the diagnosis of pheochromocytoma or paraganglioma. Interestingly, we also identified a germline mutation, D119E, in the SDHD gene (fig 4). Two common polymorphisms were also found, T891C in SDHA and C18A in SDHB.

Five years after the nephrectomy, the patient is alive with continuing disease. He had an enlarging para-aortic mass without hypertension or elevation of any catecholamine related markers in blood and urine. Strong adhesion around the duodenum and inferior vena cava prevented further surgical resection of the residual tumour. After a diagnosis of
malignant intrarenal pheochromocytoma was established, the patient underwent external beam radiation therapy, delivering 50 Gy to the residual para-aortic tumours. One month after radiation therapy, MRI revealed a 25% reduction in the para-aortic masses.

DISCUSSION

Previously, in a microarray study of acute leukaemia, one particular case showed a gene expression profile that fitted neither acute myeloid leukaemia (AML) nor acute lymphoblastic leukaemia. This brought into question the diagnosis of that case as AML. Subsequent analysis found that the tumour had high expression of certain particular genes suggesting a mesenchymal origin. Further cytogenetic study, which revealed a pathognomonic translocation, confirmed it as an alveolar rhabdomyosarcoma, and the treatment for the patient was revised accordingly.

Similarly, our microarray based molecular classification study of RCC revealed that a previous diagnosis of RCC was actually a rare malignant intrarenal pheochromocytoma. This change in diagnosis was only possible because of the gene expression microarray. The molecular profile of this case clearly did not fit with any of the other RCC cases, leading to further investigation by immunohistochemical staining and mutation analysis. Using normal kidney tissues as a control, this study of RCC revealed that a previous diagnosis of RCC was actually a rare malignant intrarenal pheochromocytoma. This case illustrates the importance and clinical implications of microarray expression profiling.

CONCLUSION

We report a very rare case of intrarenal pheochromocytoma in a 26 year old man whose final diagnosis was first suspected because of microarray gene expression profiling. This then led to further immunohistochemical staining for neuroendocrine markers and mutation analysis of the known paraganglioma associated genes, which revealed an unexpected predisposing germline mutation. This case illustrates the importance and clinical implications of microarray expression profiling.

ACKNOWLEDGEMENTS

This work was supported by the Fischer Family Trust Foundation, the Hauenstein Family Foundation, and the Van Andel Research Institute. We would like to thank S Antio for assistance with manuscript preparation and submission.

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Figure 3 Clustering of 70 kidney tumours. The clustering of patients (using Pearson’s correlation) is based on global gene expression profiles. The tumours clustered into two broad groups with one group consisting of primarily clear cell RCC and the other consisting of all other kidney tumours. Five chromophobe RCCs and two oncocytomas are clustered close together. Eight papillary RCCs, five Wilms tumours, and three TCC are clustered together as groups. The case “granular 5,” which has its own distinct molecular signature, does not cluster with any other type of kidney tumour.

Figure 4<br>Germline D119E (exon 4) mutation; the succinate dehydrogenase subunit D (SDHD) gene.
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Received 4 February 2005
Revised 22 March 2005
Accepted 24 March 2005

REFERENCES


